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The Participation of the Anterior Chamber of the Eye in Resistance Phenomena Related to Tumor Growth*

Harry S. N. Greene, M.D.

(From The Rockefeller Institute for Medical Research, Princeton, N. J., and the Department of Pathology, Yale University School of Medicine, New Haven, Conn.)

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The ease with which tumors and other living tissues can be transplanted from one animal to another by utilizing the anterior chamber of the eye as an inoculation site has given rise to speculation concerning the nature of the factors determining the increased susceptibility of the chamber as compared with other bodily regions.

The observations of Becht and Greer (1), Hektoen and Carlson (6), and others, who found a very low concentration or complete absence of antibodies in the aqueous humor of immunized animals, suggested that a barrier might exist between the blood and aqueous humor which prevented the passage of antibodies into the chamber. Besredka and Bardach (2), however, were unable to transplant the Brown-Pearce tumor in the anterior chamber of rabbits "immunized" to that tumor, which indicated either that a barrier to "tumor antibodies" did not exist or that the resistance to tumor reinoculation was different in nature and mechanism from the immunity following the injection of bacterial and other foreign proteins. On the other hand, Saphir, Appel, and Straus (7), working with the same tumor in "immune" rabbits, have recently reported that, after a latent period, growth occurred in the anterior chamber whereas other tissues resisted transplantation. In an attempt to clarify this question data obtained from reinoculation experiments involving other rabbit tumors have been analyzed and are presented in this report.

MATERIALS AND METHODS

The tumors used in these experiments included H31, an adenocarcinoma of the uterus (3, 4), T36, an acinar type of breast cancer (5), and B240, a papillary type of breast cancer (5).

The growth characteristics of these tumors have been studied by long serial passage in normal rabbits and have been described in the papers referred to above.

It is necessary to note several of their distinctive features, however, for a proper interpretation of the experiments. The T36 tumor grows rapidly to fill the anterior chamber in from 10 days to 2 weeks, and its vigorous proliferation is followed by equally rapid regression so that, within 3 weeks of transfer, the tissue is completely necrotic. The transplants invariably undergo regression after filling the chamber, and in no instance have metastases been found nor has it been possible to transfer to other bodily regions. The H31 tumor grows more slowly and rarely fills the chamber in less than a month. Regression occurs in approximately 40 per cent of cases but proceeds slowly and living tissue persists for 6 to 8 weeks. In other instances, growth is progressive and terminates in metastasis. Testicular transfer is successful in a high percentage of cases and growth is almost invariably progressive. The B240 tumor maintains the slowest growth rate of the 3 tumors and usually requires 2 or more months to fill the chamber. Its subsequent course is one of partial regression with alternating periods of renewed growth and may extend for a year or more without metastasis. Testicular transfer is only rarely successful.

The procedure employed in the present experiments consisted of a preliminary anterior chamber transfer with subsequent reinoculation in the other chamber after varying periods of time. In several instances the testicle rather than the eye was used as a site of primary transfer, while in others the presence or absence of generalized susceptibility was tested by reinoculation in this organ. The development of resistance to reinoculation was determined both in relation to the tumor used in the original transfer and in relation to an entirely different tumor. The experiments were controlled throughout by coincident transfer to normal animals.

RESULTS

In the first experiment of this series fragments of the H31 tumor derived from a rabbit of the 7th serial eye generation were transferred to the anterior chambers of 13 normal animals of the same strain, all of which were within 2 weeks of 7 months of age. Takes occurred in all instances and the transplants

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grew to fill the chambers in approximately 3 weeks. Regression followed and at the time of the first reinoculation experiment, 167 days after the original transfer, the necrotic remains of the transplants had been completely resorbed.

The results obtained on reinoculation of these rabbits are shown in Table I. No takes occurred from transfer of the H31 tumor to the left eye on the 167th or 337th days and, in like manner, testicular transfer on the 400th day resulted in no growth. Attempts to transplant the T36 tumor to the left eye on the 203rd and 230th days were also unsuccessful. In contrast, transfer to normal animals gave rise to a high percentage of takes in all cases.

Additional attempts were made to transfer to the left eye at shorter intervals after primary growth in the right eye, and the results obtained by using the same tumor for both transfers are shown in Table II.

TABLE I: THE RESULTS OF REINOCULATION IN THE ANTERIOR CHAMBER OF THE OPPOSITE EYE AND IN THE TESTICLE

Days after successful transfer of H31 to right eye		167	203	230	337	400
Tumor used for reinoculation		H31	T36	T36	H31	H31
Site of reinoculation		Left eye	Left eye	Left eye	Left eye	Right testicle
Number of controls		6	5	6	10	7
Takes in controls, per cent		100	80	100	100	71.4
Rabbit No.	Sex					
1	M	0	..	..	0	..
2	M	0	..	..	0	0
3	M	0	..	..	0	0
4	M	0	..	..	0	0
5	M	0	..	..	..	0
6	M	0	..	..	..	0
7	F	0	0	0	..	..
8	F	0	0	0	0	..
9	F	0	0	0	..	..
10	F	0	0	0	..	..
11	F	0	0	0	..	..
12	F	0	0	0	..	..
13	F	0	0	0	..	..

0 indicates no growth.

TABLE II: THE RESULTS OF REINOCULATION OF THE SAME TUMOR IN THE ANTERIOR CHAMBER OF THE OPPOSITE EYE

Tumor and inoculation site				Reinoculation				Control inoculation		
1st inoculation		Reinoculation				Takes			Takes	
Tumor	Site	Tumor	Site	Days after 1st inoculation	Number of rabbits	Number	Per cent	Number of rabbits	Number	Per cent
T36 *	Right eye	T36	Left eye	16	1	0	0	6	6	100
"	" "	"	" "	23	4	0	0	5	5	100
"	" "	"	" "	24	2	0	0	6	6	100
"	" "	"	" "	28	1	0	0	6	6	100
"	" "	"	" "	33	1	0	0	5	5	100
"	" "	"	" "	35	1	0	0	5	4	80
"	" "	"	" "	37	4	0	0	7	7	100
"	" "	"	" "	40	3	0	0	5	5	100
"	" "	"	" "	42	4	0	0	5	4	80
		Total			21	0	0	50	48	96
H31 †	Right eye	H31	Left eye	167	13	0	0	6	6	100
"	" "	"	" "	337	5	0	0	10	10	100
		Total			18	0	0	16	16	100
B240	Right eye	B240	Left eye	24	9	6	66.6	9	8	88.8
"	" "	"	" "	41	1	0	0	4	2	50
"	" "	"	" "	56	1	1	100	4	2	50
		Total			11	7	63.6	17	12	70.5

* The original transplant in the right eye was alive and growing at the time of reinoculation.
† Summarized from Table I.

The T36 and B240 tumors were utilized in this experiment primarily to contrast the resistance-provoking action of a rapidly growing, short-lived tumor on the one hand with a slowly growing, long-lived tumor on the other. It should be noted that, in the case of the T36 tumor, regression of the primary transplant was complete or became complete shortly after reinoculation, whereas in all instances the original B240 transplants contained living tumor tissue and complete regression did not occur for a considerable period of time thereafter.

Reinoculation with the T36 tumor was uniformly unsuccessful and resistance to transfer was present even before regression of the original transplant had occurred. In contrast, reinoculation with the B240 tumor resulted in almost as high a percentage of takes as was observed in normal controls.

The animals bearing B240 transplants were held for continued observation and some interest attaches to the finding that regression of the tumors occurred simultaneously in both eyes, despite the fact that the 2nd transplant had often grown to fill only a quarter of the chamber. Four animals of this class were subjected to a 3rd inoculation of the tumor and, although the small number precludes final judgment, it is suggestive that no takes occurred.

Table III presents the results of attempts to reinoculate animals in the left eye with a tumor different from that originally grown in the right eye.

As may be noted, the incidence of takes of H31 in animals previously inoculated with T36 was considerably less than that observed in normal controls. A significant reduction in the number of takes resulted also from transfer of T36 to animals originally inoculated with H31. In the latter case takes occurred up to the 68th day, but reinoculation thereafter was uniformly unsuccessful. It should be emphasized that, previous to this time, transplants of the H31 tumor normally contain intact cells which are viable and capable of proliferation on transfer to fresh animals. This, however, does not apply to transplants of the T36 tumor, in which all cells are invariably dead and necrotic by the end of the 3rd week. Thus complete regression of the H31 tumor appears in all cases to be associated with resistance to transplantation of T36, but the reverse does not hold, and regression of T36 may or may not confer protection against subsequent transfer of H31.

In all cases in which reinoculation was successful, the latent period between transfer and the appearance of signs of growth was considerably increased over that observed in controls. In addition, growth was slow and regression delayed. The T36 tumor resulting from reinoculation showed no histological alteration, but a consistent change was noted in the architecture of the H31 tumor. In normal animals this tumor forms solid medullary masses with relatively little glandular structure and scanty stroma. However, in animals that have previously borne T36 it manifests a pronounced adenoid tendency with the production of numerous, well formed glandular acini, and hyalinization of the stroma is common.

A relatively small number of animals were used in attempts to reinoculate the B240 tumor after growth

TABLE III: THE RESULTS OF REINOCULATION OF A DIFFERENT TUMOR IN THE ANTERIOR CHAMBER OF THE OPPOSITE EYE

Tumor and inoculation site				Reinoculation				Control inoculation		
1st inoculation		Reinoculation				Takes			Takes	
Tumor	Site	Tumor	Site	Days after 1st inoculation	Number of rabbits	Number	Per cent	Number of rabbits	Number	Per cent
T36	Right eye	H31	Left eye	35	2	1	50	7	7	100
"	" "	"	" "	45	1	0	0	7	7	100
"	" "	"	" "	56	1	1	100	7	7	100
"	" "	"	" "	60	4	1	25	6	6	100
		Total			8	3	37.5	27	27	100
T36	Right eye	B240	Left eye	29	3	0	0	4	2	50
"	" "	"	" "	46	3	0	0	4	2	50
		Total			6	0	0	8	4	50
H31	Right eye	T36	Left eye	27	5	2	40	5	5	100
"	" "	"	" "	38	5	3	60	6	6	100
"	" "	"	" "	41	1	0	0	5	4	80
"	" "	"	" "	68	3	1	33.3	5	5	100
"	" "	"	" "	69	1	0	0	6	6	100
"	" "	"	" "	104	1	0	0	5	4	80
"	" "	"	" "	203	7	0	0	5	4	80
"	" "	"	" "	230	7	0	0	6	6	100
		Total			30	6	20	43	40	93

of T36 but the results were consistent. In this case, growth of T36 appears to have afforded complete protection against B240 despite the fact that in previous experiments the state of resistance produced by this tumor was found insufficient to prevent growth of H31.

Further attempts to study resistance relationships during active tumor growth were carried out by transferring the H31 and B240 tumors to animals bearing testicular transplants of H31. The H31 tumor grows slowly in the testicle and, in the great majority of cases, persists for a year or more to terminate in metastasis. Complete regression is rarely observed but localized areas of necrosis and fibrous tissue replacement are a constant feature.

Table IV: The Results of Reinoculation in the Anterior Chamber after Previous Growth in the Testicle

Tumor and inoculation site				Reinoculation				Control inoculation		
1st inoculation		Reinoculation				Takes			Takes	
Tumor	Site	Tumor	Site	Days after 1st inoculation	Number of rabbits	Number	Per cent	Number of rabbits	Number	Per cent
H31	Right testicle	H31	Right eye	50	5	1	20	6	5	83.3
"	" "	"	" "	82	4	1	25	6	5	83.3
"	" "	"	" "	88	1	1	100	3	3	100
"	" "	"	" "	121	2	0	0	5	3	60
"	" "	"	" "	123	2	1	50	6	6	100
"	" "	"	" "	126	2	2	100	5	3	60
"	" "	"	" "	135	2	1	50	5	3	60
"	" "	"	" "	146	1	0	0	3	3	100
"	" "	"	" "	181	1	0	0	6	6	100
		Total			20	7	35	45	37	82.2
H31	Right testicle	B240	Right eye	47	3	0	0	3	3	100
"	" "	"	" "	57	1	0	0	3	3	100
"	" "	"	" "	70	1	0	0	3	3	100
		Total			5	0	0	9	9	100

The outcome is presented in Table IV. Reinoculation of the same tumor (H31) resulted in a considerably reduced incidence of takes as compared to normal controls. It would appear, therefore, that the development of resistance to reinoculation is not dependent on complete regression of the primary tumor but may take place in the presence of intact living cells, and the same situation obtains whether the living tumor cells are located in the eye or in the testicle.

A degree of resistance was also manifest in instances where takes occurred and the transplants were modified both in behavior and morphology. The latent period was increased, growth was slow, and the histological picture closely resembled that found in transplants of the same tumor following growth of T36 (Figs. 1 and 2).

Reinoculation of the B240 tumor in the presence of growing transplants of H31 gave rise to no takes. Such results are in line with previous experience with this tumor, and further demonstrate its increased sensitivity to altered constitutional states and its inability to grow at all in environments offering only partial resistance to the growth of other tumors.

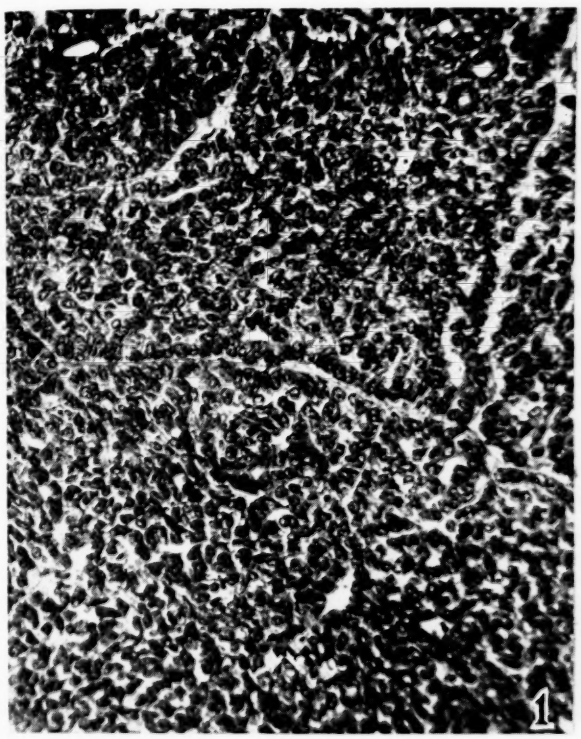
DISCUSSION

The primary object of these experiments was to determine whether the anterior chamber of the eye shared in the general bodily reactions concerned with acquired resistance to tumor transplantation, or whether a barrier existed which isolated the aqueous humor from the remainder of the organism. The method consisted of reinoculation attempts carried out at various periods after primary tumor growth in the anterior chamber, and the results were interpreted on the assumption that the existence of a blood-aqueous humor barrier would prevent the occurrence of a generalized resistance to transplantation such as usually follows tumor growth in other bodily regions. The majority of reinoculation experiments were performed in the anterior chamber of the opposite eye which, on a basis of the barrier theory, should be doubly separated from the primary inoculation site.

It was found that, following tumor growth in the anterior chamber, both the testicle and the anterior chamber of the opposite side were resistant to reinoculation. The degree of resistance varied, but the variation was no greater than is constantly observed in reinoculation experiments involving primary growth in other bodily regions (8). The conclusion appears justified, therefore, that the aqueous humor is not an independent, isolated fluid but, on the other hand, participates in general bodily reactions, at least as far as tumor resistance is concerned.

In point of fact, the anterior chamber is an ideal

site for the study of tumor resistance, for here the results of both primary and secondary inoculations can be noted directly, takes can be determined without recourse to biopsy, time relations and growth rates can be accurately measured, and a variety of similar observations made without uncertainty or the necessity of sacrificing the animal with the attendant danger of premature termination of the experiment. Moreover, the chamber offers a more sensitive test for resistance and, because of its high susceptibility to transfer, supports growth during states in which other bodily regions appear refractory. The ability to support growth during states of partial resistance is observed in natural as well as acquired forms, and appears to be related to local conditions favoring growth rather than to a failure of the chamber to share in general bodily reactions.



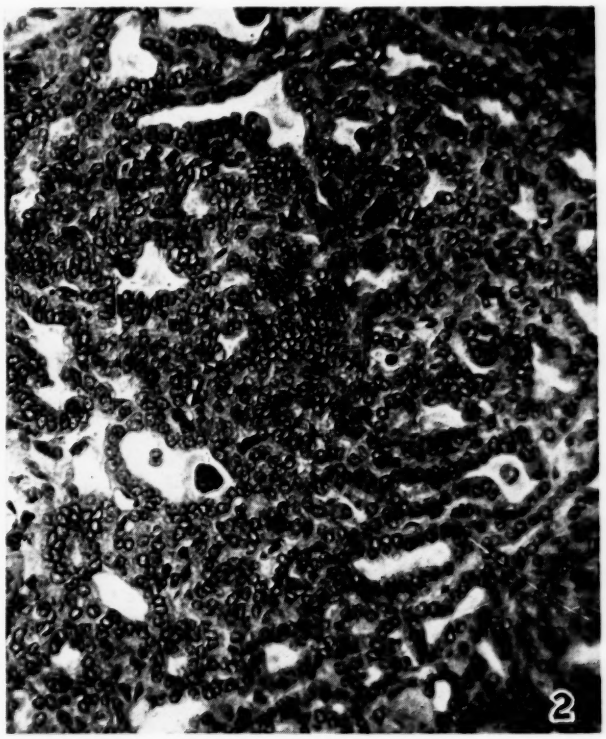


FIG. 1.—Section of transplant of H31 tumor from the anterior chamber of a normal rabbit, showing typical medullary form of growth. Hematoxylin and eosin. Mag. × 250.

FIG. 2.—Section of transplant of H31 tumor from the anterior chamber of a rabbit bearing a testicular growth of the same tumor. There is a decided tendency to form adenoid structures which contrasts with the medullary type of growth found in normal animals. Hematoxylin and eosin. Mag. × 250.

An interpretation of the results obtained based on the conclusion that the anterior chamber of the eye shares in general body reaction, brings out several points of interest relative to acquired tumor resistance. Moreover, the behavior of the various tumors in relation to resistance phenomena can be correlated with known facts pertaining to their growth characteristics and morphology.

The tumors used in the experiments were selected because of distinctive differences in growth rate, longevity, and morphology. B240 is a papillary type of tumor requiring an abundant stroma in intricate arrangement, and apparently the stromal requirement is obligatory, for growth has never been observed in the rabbit or in an alien species without the characteristic structural pattern. H31 requires only a minimum of stroma to maintain itself in the medullary form found in normal rabbits of the majority of breeds. However, several breeds, notably the Himalayan as well as a number of foreign species, possess a degree of natural resistance to the tumor and here the stromal reaction is altered and results in a pronounced adenoid architecture. In contrast, the T36 tumor requires only a simple connective tissue scaffolding to support its growth and, while structural variations are found in alien hosts, variations do not occur in the rabbit. The growth rates of the tumors are inversely proportional to their stromal requirement and longevity appears to bear a similar relationship.

It was observed that complete regression of any of these tumors was associated with the development of a state of solid resistance to reinoculation of the same tumor in other bodily regions, and in the case of the H31 tumor this state was found to persist for at least 400 days. Resistance to tumors different from those

used in the primary transfer was also apparent on reinoculation but, as might be expected, the degree of resistance evoked was generally less than that directed against growth of the same tumor.

States of partial resistance were found to characterize animals bearing living tumors, and experiments carried out during this period revealed relationships which were overwhelmed and obscured in states of complete resistance. It was noted, for example, that the degree of resistance evoked varied with the tumor used and bore a direct relationship to the rate and duration of growth. Thus in the case of the rapidly growing, short-lived T36 tumor, complete resistance to reinoculation was present even during late stages of active growth of the primary transplant. Partial resistance, evidenced by reduced takes, slow growth, and altered architecture characterized the growing period of H31, whereas no indication of resistance whatsoever was observed in animals bearing living cells of the slowly growing, long-lived B240 tumor. Continued residence of the growing tumor in the body, or regression, was associated with an increased degree of resistance, and in the case of the H31 tumor this was found to embrace increased resistance to T36 as well. Here partial resistance only was present during early growth stages, while complete resistance followed prolonged growth in the testicle or regression in the anterior chamber.

A variation was noted also in the ability of tumors to grow during states of incomplete resistance, and this appeared to be related to the stromal requirements of the tumor. Thus H31 was able to grow after regression of T36 (37.5 per cent of takes in contrast to 100 per cent in controls), while, under the same conditions, the more highly organized B240 failed completely. In like manner T36 grew in animals bearing active transplants of H31, whereas attempts to transfer B240 under similar conditions were entirely unsuccessful.

It is generally agreed that stroma production is a host reaction to implanted tumor cells, and it is not unreasonable to assume that a modification of the host, such as follows previous tumor growth, might result in a variation in stroma formation. Conceivably, then, the modified reaction might be of such an order as to prevent the production of the specialized stroma needed for growth of B240 and yet allow production of the comparatively simple connective tissue scaffolding required by T36 and H31. Such a conception is in line with the observed behavior of these tumors. Moreover, the stroma supplied for the growth of the H31 tumor by partially resistant animals was actually abnormal in quality as evidenced by hyaline transformation. It is suggested, therefore, that the ability of a tumor to grow in resistant environments is a function of its stromal requirement. When the requirement is slight the host response may be sufficient or, if the tumor is capable of structural variation, it may adapt itself to a new and limited stromal allowance. On the other hand, if a specialized stroma is obligatory, or if the degree of resistance prohibits any stromal response, growth does not occur and the host is said to be refractory to the tumor.

SUMMARY

A series of experiments was performed in an attempt to ascertain whether or not a blood-aqueous humor barrier separated the anterior chamber of the eye from the remainder of the body, and thus determined the ease with which tumor transplantation was effected at this site. It was found that, following tumor growth in the anterior chamber of one eye, both the testicle and the anterior chamber of the opposite side were resistant to reinoculation. On this basis it was concluded that the aqueous humor was not an isolated fluid but participated in general body reactions, and that its susceptibility to tumor transfer was not determined by an antibody barrier.

An interpretation of other reinoculation experiments, based on this conclusion, suggested that the resistance-provoking powers of the tumors used were directly related to their growth rates and longevity. The ability of the various tumors to grow in partially resistant hosts appeared, on the other hand, to be a function of their stromal requirements.

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Mechanism of Tumor Immunity as Investigated by Means of the Intraocular Inoculation of the Brown-Pearce Carcinoma

F. S. Cheever, M.D., and Herbert R. Morgan, M.D.*

(From the Department of Bacteriology and Immunology, Harvard Medical School and School of Public Health, the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, Boston, Mass.)

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The mechanism of the immunity which can be induced in rabbits against the highly malignant Brown-Pearce carcinoma remains obscure.

Following the regression of intracutaneous implants of tumor tissue, Besredka and his collaborators (3-5) were unable to demonstrate the existence of humoral antibodies against the tumor. These observations were confirmed and extended by Gross (9) and by Cheever and Janeway (6). Furthermore, Favorite and Cheever (7) did not observe any deleterious effect on tumor cells growing in roller tube tissue cultures when sera and tissue extracts from immune rabbits were added. The pathological studies so far reported (1, 6) have failed to elucidate the problem.

Greene's (8) success in transferring homologous and heterologous tumors into the anterior chamber of the rabbit's eye suggested that this technic might offer certain advantages for the analysis of the immune mechanism, especially as Besredka and Bardach (2) had failed to obtain takes in resistant animals following intraocular implantation. Although the original purpose of the present investigation was to determine whether the tumor cells actually succumbed in the aqueous humor of the immune animal or merely remained inactive but viable, the recently reported failure of Saphir and his collaborators (12) to confirm the results of the French workers led us to reinvestigate the entire problem.

MATERIALS AND METHODS

A hybrid lot of brown-gray and white rabbits were used. The routine preparation and propagation of the tumor have been described (6). The methods of immunization were essentially those described by Pearce and Brown (11) and further developed by Besredka and his collaborators (3). The technic of intraocular inoculation closely followed that described by Krichesky and his collaborators (10). Each batch of tumor material used for challenging inoculations (*i.e.* to determine the presence or absence of immunity against the carcinoma) was tested for viability by the inoculation of a portion into the anterior chamber of the eye of one or more normal animals; in all cases typical takes resulted.

Primary inoculations.—To test the validity of the experimental procedure, 29 rabbits were inoculated intraocularly with tumor fragments. Seven of these animals received intratesticular inoculations also at the same time. The results are presented in Table I.

In the cases of successful grafts, growth was usually obvious at the end of a week, and the subsequent proliferation of the tumor was sufficiently rapid to fill the entire orbit within a month. The animals were sacrificed at this time. No metastases were found at autopsy. Only once was regression of an apparently well established growth observed: one rabbit receiving implants simultaneously in both eyes showed bilateral proliferation for the first 10 days followed by spontaneous regression of both tumors.

TABLE I: PRIMARY INOCULATIONS OF NORMAL ANIMALS

Number of rabbits inoculated	A. One eye inoculated: Takes	A. One eye inoculated: Failures to take
14	12	2

Number of rabbits inoculated	B. Both eyes inoculated simultaneously: Takes in both eyes	Takes in one eye	Failures to take
8	5	2	1

Number of rabbits inoculated	C. One eye and one testicle inoculated simultaneously: Takes in testicle and in eye	Takes in eye only	Takes in testicle only	Failures to take
7	7	0	0	0

These preliminary experiments confirmed previous observations that unilateral or bilateral intraocular inoculations of the Brown-Pearce carcinoma in normal animals gave rise to progressive growths in at least 85 per cent of the cases, that regression was rare, and finally that gross metastasis did not take place within a month. Simultaneous intraocular and intratesticular inoculations gave rise to progressive growths at both sites.

Secondary eye inoculations.—Sixteen animals received inoculations of the tumor material in one eye.

* John Ware Memorial Fellow.

At varying periods thereafter fresh tumor material was introduced into the other eye and the fate of this 2nd graft observed.

Tumors growing in one eye for as long as 3 weeks were usually ineffective in preventing the growth of carcinomatous material subsequently implanted in the other eye. Takes resulted in all but 4 out of 17 animals tested. There was no evidence that the state of the first tumor was of importance in affecting the fate of the 2nd graft in 76 per cent of the animals tested.

Rabbits were then immunized by several of the methods referred to above (6) and at varying intervals thereafter each was given an intraocular inoculation of viable tumor material. In addition to these methods, several animals from which growing tumors were removed by operative procedures and a group with

Removal of tumor from eye of immunized animals for reimplantation.—It was noticed in the previous experiments that intraocular implants, while failing to grow and becoming pale and slightly shrunken in the anterior chambers of the eyes of immune rabbits, did not become necrotic or disintegrate for long periods of time. Accordingly it became of interest to determine whether these fragments were merely lying dormant or whether some agent in the aqueous humor (antibody?) had caused the cells to lose their property of malignant proliferation. Rabbits immunized by the regression of testicular or intracutaneous tumors were inoculated in one eye with carcinomatous material as a test for immunity. An equal number of normal animals were inoculated simultaneously and typical takes were observed in all this latter group, but none

TABLE II: SECONDARY EYE INOCULATIONS

Rabbit No.	Primary inoculation		Time between primary and secondary inoculation, days	State of implant in 1st eye at time of inoculation of 2nd eye	2nd inoculation		Time between 2nd inoculation and autopsy, days
	Eye	Result			Eye	Result	
60	Left	Take	4	Definite take with increase in size	Right	Take	22
62	"	"	5	No activity	"	"	27
65	"	"	5	Definite take with increase in size	"	"	27
67	"	"	6	No activity	"	"	10
68	"	"	6	" "	"	"	21
369	"	"	6	" "	"	"	26
79	Right	No take	7	" "	Left	No take	38
156	"	Take	8	Definite take with considerable growth	"	" "	43
70	Left	"	12	Definite take, fragment tripled in size	Right	Take	15
69	"	"	12	" " " " " "	"	"	29
76	"	"	13	Anterior chamber half filled	"	No take	35
77	"	"	13	Definite take, fragment tripled in size	"	Take	35
400	Right	"	15	Anterior chamber $\frac{1}{4}$ filled	Left	"	20
73	Left	"	21	Anterior chamber filled	Right	"	14
74	"	"	21	Anterior chamber $\frac{2}{3}$ filled	"	"	14
75 *	"	"	21	Anterior chamber $\frac{3}{4}$ filled	"	No take	14
219	Right	"	120	Anterior chamber filled	Left	Take	14

* Received a bilateral testicular inoculation at same time. Bilateral tumors resulted.

proliferating tumors in various sites were tested for susceptibility by intraocular tumor implants. The results are summarized in Table III.

The presence of a growing tumor in the testicle or skin prevented the growth of a tumor in the eye in all but 1 of 5 animals so treated. The spontaneous regression or the surgical removal of previously implanted tumors appeared to render 90 per cent of rabbits used in this experiment refractory to subsequent intraocular inoculations of the carcinoma. In 4 instances (rabbits No. 51, 95, 64, 339) unsuccessful attempts were made to break down this immunity by vascularizing the cornea to provide a rich capillary bed, previous to the implantation of the tumor in the anterior chamber of the eye.

From these results one may conclude that all methods of immunization used appeared to be equally effective.

among the immune rabbits although they were carefully observed for 6 weeks. The animals were then subjected to a 2nd intraocular inoculation with fresh tumor material in the opposite eye. Again no takes were obtained, although portions of the same malignant tissue introduced simultaneously into the eyes of normal rabbits gave rise to typical proliferative lesions in every instance.

After remaining for varying periods of time (5 to 14 days) in the anterior chambers of the eyes of the immune rabbits, these fragments of tumor material were removed and introduced into the eyes of normal rabbits. At the time of transfer the fragments appeared quiescent; they were pale and slightly shrunken but of relatively firm consistency with no evidence of either necrosis or inflammatory reaction. These fragments (which were somewhat larger than those employed in the previous experiments) gave rise to typical

takes in some animals but in others showed no sign of activity during the 6 week period of observation. The susceptibility of these rabbits to the tumor was proved by the subsequent inoculation of fresh malignant tissue into the anterior chamber of the other eye. In each case a typical take resulted.

The procedures employed in these experiments will be apparent from the data included in Table IV. The results are summarized in Table V.

TABLE III: INTRAOCULAR INOCULATIONS IN IMMUNIZED ANIMALS

Rabbit No.	Route and method of immunization	Interval between immunizing and test inoculations, days	Test inoculation: Eye	Test inoculation: Result	Interval from test inoculation to autopsy, days
GROUP I	Regression of testicular tumor				
51		90	Left	No take	90
21		40	Right	" "	48
95		109	Left	" "	90
385		50	"	" "	60
GROUP II	Regression of intraperitoneal tumor				
34		54	Left	No take	56
			Right	" "	
GROUP III	Regression of testicular and intraperitoneal tumors				
50		91	Left	No take	60
48		58	"	" "	41
			Right	" "	
41B		49	Left	Take followed by regression	51
			Right		
39B		99	Left	No take	41
			Right	" "	
98		145	Left	" "	54
			Right	" "	
GROUP IV	Regression of intracutaneous tumors				
339		57	Right	No take	90
402		49	Left	" "	
			Right	Early take, subsequent regression	60
348B		14	Left	No take	37
GROUP V	Excision of testicular tumor 14 days previous to test inoculation	154	Left	" "	32
346	Excision of testicular tumor day of test inoculation	14	Left	" "	32
344	Testicular tumor	14	"	" "	32
348	" "	14	"	" "	32
281	" "	7	"	" "	25
282	" "	7	"	" "	25
206	5 intracutaneous tumors	14	"	Take	26

TABLE IV: REIMPLANTATION OF TUMOR FRAGMENTS FROM EYES OF IMMUNIZED ANIMALS

Rabbit No.	Date immunized, 1941	Method of immunization	Date of test of immunity, 1941	Test inoculation: Eye	Test inoculation: Result	2nd intraocular implant: Eye	2nd intraocular implant: Number of days in eye of immune animal	Transplant to normal animal: Rabbit No.	Transplant to normal animal: Result	Test of susceptibility of normal rabbit: Result
385	Jan. 23	Regression of testicular tumor	Mar. 13	Left	No take	Right	14	206	No take	Typical take
348B	Aug. 8	Regression of intracutaneous tumors	Aug. 18	Right	" "	Left, 2 fragments	10	292	Take	.....
								293	"	.....
350	Nov. 15	" "	Nov. 29	Left	" "	Right, 2 fragments	14	1421	No take	.....
								219	Take	.....

From 3 of the animals with reimplanted fragments of tumor which had resided in the eye of an immune animal for 14 days, fragments were taken for micro-

scopic examination 4 months following the reimplantation. The fragments at this time were pale and slightly shrunken but not necrotic. Study of the stained sections revealed that the tumor cells were replaced by a mass of collagenous material containing a few capillaries and occasionally an isolated tumor cell or a small group of such cells which showed no signs of proliferation. This experiment suggested a possible relationship between the increasing length of time a tumor fragment remained in the eye of an immune animal and its decreasing chance of proliferating when transferred into a normal rabbit. That the actual transfer of tumor material from one eye to another did not affect its chances of proliferation in the 2nd animal was shown by observations that fragments growing in normal rabbits as long as 10 and 14 days, and up to 3 months in one case, always continued to proliferate when transferred to other normal animals.

TABLE V: SUMMARY OF REIMPLANTATION OF TUMOR FRAGMENTS

Number of days tumor fragments remained in eyes of immunized rabbits	Number of fragments inoculated into eyes of normal rabbits	Number of takes	Number of failures to take
5	6	3	3
10	6	2	4
14	11	2	9

DISCUSSION

Besredka and Bardach (2) failed to engraft the Brown-Pearce carcinoma in the eyes of rabbits previously immunized against the tumor. Our experiments bear out their observations, and are not in accord with those of Saphir and his coworkers (12). These investigators suggest that the French workers did not extend their observations for a sufficient period, but this explanation of the discrepancy does not seem applicable to the present experiments since the great majority of our animals were not killed until at least 6 weeks or more had elapsed after the test inoculations.

A growing testicular tumor immunized against subsequent intraocular inoculations, but a proliferating carcinoma in one eye failed to render the animal refractory to subsequent inoculations of tumor material into the other eye. Intraocular tumors appeared to be exceptions to the general rule that proliferating growths immunize the animal against subsequent inoculations of the same material. The reason for this is not clear, but the failure of Greene (8) and of the present authors to discover metastases from anterior chamber tumors may have some relation to this lack of immunity since the failure of tumor cells to escape from the eye may prevent the inauguration of a general systemic resistance which is dependent upon some hypothetical immunizing agent in such material.

By what mechanism is the proliferation of tumor grafts in the anterior chamber of the eyes of immunized rabbits prevented? The implanted fragments remained intact for several weeks, showing no signs of necrosis. Ultimately they became pale and somewhat shrunken but we never observed any evidence of an inflammatory reaction in either iris or cornea; in fact these structures in the immune animal appeared indifferent to the presence of the malignant cells. There was no indication of a foreign body reaction such as has been described surrounding regressing tumors implanted elsewhere (13). The microscopic examination of 3 of these tumor fragments, which on removal from the immune host were reimplanted in a normal animal, likewise showed no evidence of necrosis or inflammatory reaction though the tumor cells were largely replaced by connective tissue including large amounts of collagen.

The failure of the host to develop an angiofibrous supporting stroma for the tumor is a possible explanation for the inability of the latter to take. Working with the rabbit carcinoma H31 Greene (8), however, has shown that in the eyes of guinea pigs the tumor may lie latent for as long as 120 days before proliferating. During this period there is no reaction on the part of the iris and no obvious blood supply to the tumor. He also noted that fragments remaining inert for over 500 days showed typical and rapid proliferation when removed from the guinea pig and transplanted into its native host, the rabbit.

Fragments of the Brown-Pearce carcinoma remaining but 5 days in the anterior chamber of the eyes of immune animals showed a 50 per cent mortality, and only 2 fragments out of 11 proliferated upon reimplantation into normal rabbits' eyes after a sojourn of 2 weeks in the anterior chambers of the eyes of immune animals. Although these findings are admittedly inconclusive they suggest the existence of an agent in the aqueous humor of the eyes of immune animals which acts slowly but nonetheless with deleterious effect upon specific tumor grafts. Its potency is probably not great since 2 fragments were able to proliferate upon transfer into normal animals after 2 weeks' contact with it in the anterior chamber of the eyes of immune animals. However, these tumor fragments were of considerable size so that malignant cells in the center of the mass were protected by adjacent cells from any agent diffusing into the piece of tumor tissue.

SUMMARY

1. The implantation of fragments of the Brown-Pearce carcinoma into the anterior chamber of the eye

of normal animals was followed by successful takes in most instances, regardless of whether the inoculation was unilateral or bilateral.

2. Secondary intraocular inoculation in animals immunized by any one of several standard methods was unsuccessful in 18 of 20 cases.

3. Secondary intraocular inoculations as long as 4 months after the primary implantation of tumor into the other eye were generally successful, regardless of the state of activity of the first growth at this time.

4. Of 11 tumor fragments remaining as long as 14 days in the eyes of immune animals only 2 proved viable and capable of delayed but sustained proliferation when implanted into the eyes of normal animals.

5. This evidence, though far from unequivocal, suggests the existence of an agent in the aqueous humor of immune rabbits' eyes capable of destroying or rendering nonmalignant the cells of the Brown-Pearce carcinoma.

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Investigation into the Possible Carcinogenic Activity of Wood Smoke

Frank Dickens, Ph.D., and Hans Weil-Malherbe, M.D.

(From the Cancer Research Laboratory, North of England Council of the British Empire Cancer Campaign, Royal Victoria Infirmary, Newcastle-upon-Tyne, England)

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In view of the great geographical variation in the incidence of gastric cancer many authors have suggested that extrinsic factors, and among them chiefly dietary habits, may contribute to the onset of this disease. Our attention has been directed by Dr. L. Weil towards the possibility that the habitual consumption of smoked food may constitute such a factor. In the smoking process foodstuffs are impregnated with tarry material produced, as a rule, by the incomplete combustion of hard wood at temperatures between 400° and 500° C. It is not unreasonable to suspect a carcinogenic action by wood smoke, in view of the well known carcinogenic activity of soot from coal fires (12). It was therefore decided to test, by application to the skin of mice, the constituents of wood smoke. For this purpose it was clearly important that the samples tested should be obtained under conditions resembling as closely as possible those employed in the food-smoking industry. The generation of the right kind of smoke is a complicated process requiring considerable skill, depending as it does on the nature of the wood, the amount of moisture, and the rate of combustion. We were fortunate in having the cooperation of the Director and Staff of the Chemical Research Laboratory, Department of Scientific and Industrial Research, Teddington, who were conducting an investigation into the chemical nature of wood smoke and who supplied us with 4 fractions of condensates which contained nearly all the nongaseous products of combustion. The wood, a mixture of oak sawdust and oak shavings, was smouldered in a semi-technical-scale plant imitating a kippering kiln. The combustion was carried out in a regulated flow of air; the temperature of the combustion zone was approximately 400°. The issuing smoke passed through an elaborate absorption train which arrested all suspended matter. The installation is described in detail in a publication by Pettet and Lane (13).

No observations are recorded in the literature which bear directly on the problem under discussion, although there are a few investigations into the carcinogenic properties of various wood tars. Schreus (15) seems to be the only one who could produce epitheliomas after painting mice with wood tar (pix liquida). The report was made in the form of an oral communication and was apparently never published in full. Kotzareff and de Morsier (8) refer to unpublished observations in which papillomas appeared on the ears of 12 rabbits which had been painted with pine tar for 67 days. They further report experiments where they painted Anatolian tar (also a tar produced from resinous woods) on the buccal mucous membranes of 3 rabbits and obtained after about 60 days a few small papillomas with a tendency to regression. One dog painted with pine tar in the mouth showed an eruption of about 100 papillomas after the 88th day. Nakamura (10), on the other hand, observed no tumors after painting 30 mice with "wood tar," whereas a few developed after the animals were painted with 2 kinds of tar produced from rice polishings. Likewise, Valade (17) failed to produce tumors with tar from hard woods. In his experiments 40 mice and 2 rabbits were painted for more than 6 months with negative results. The wood tars employed in these studies are probably not entirely comparable with the tar produced in the food-smoking process. Valade alone worked with tar from hard woods, which are exclusively used for the preservation of food, whereas the other authors employed commercial samples which are as a rule prepared from resinous woods. Even Valade's tar does not correspond to that used in our experiments, as it was probably prepared by simple destructive distillation, whereas our wood smoke condensates were obtained from wood smouldering in a current of air. The products obtained by these 2 methods differ chemically (13): the product of destructive distillation contains more phenols, aliphatic acids, and formaldehyde, and less resins than that obtained by smouldering in a current of air. The resins also differ in solubility and consistency.

The investigations just reviewed all suffer from the fact that the application of the tar was discontinued after a few months, whereas a weak carcinogenic activity may have manifested itself only after a much more prolonged application.

It is of interest to note that benign and malignant tumors have been produced with condensates of tobacco smoke (14, 5). According to Flory (5) these condensates are less active than a tar prepared by destructive distillation of tobacco.

Description of the tar fractions tested.—The 4 fractions supplied to us were labeled as follows:

Fraction 1: Material extracted by ether from pyroligneous acid.

Fraction 2: Tar separated out from pyroligneous acid.

Fraction 3: Products extracted from smoke scrubbers by methylene dichloride.

Fraction 4: Products extracted by acetone subsequent to methylene dichloride extraction.

The following particulars of the preparation and the chemical properties of these fractions are taken from the paper of Pettet and Lane (13). The smoke was driven down a water-cooled condenser into an ice-cooled tank where an aqueous condensate collected. This was extracted by ether for 3 weeks in a continuous extractor. The residue, after evaporation of the ether, furnished fraction 1. It contained alcohols (mainly methyl and ethyl), aldehydes (mainly formic), and acids (formic and acetic), together with higher boiling aldehydes and phenolic and resinous material. Fraction 2 had separated out in the condenser and tank from which it was washed out by acetone. It consisted mainly of resins with aldehydic, phenolic, acidic, and neutral components. After passing the tank the smoke was led through a series of scrubbers filled with adsorbents. Fractions 3 and 4 were obtained by extracting the combined adsorbents first with methylene dichloride and then with acetone. Fraction 3 contained ketones (mainly acetone), acid, aromatic aldehydes (furfuraldehyde and 5-methylfurfuraldehyde), phenols, and resinous matter. Fraction 4 was entirely resinous.

The fractions were liquid or viscous with the exception of fraction 2, which was semisolid. They were all dark brown and had a characteristic pyroligneous odor. No fluorescence was visible in ultraviolet light even in dilute solution, but the fluorescence of added substances, such as dibenzanthracene, was completely quenched.

Methods of testing.—One hundred male market mice of mixed stock were divided into 5 groups of 20. Four of these were painted twice weekly with the 4 fractions of wood smoke, one fraction for each group of mice, the application being made to the interscapular region with a small paint brush. The fifth group served as control. The mice were maintained on a diet of brown bread and water with weekly supplements of cod liver oil, marmite, and oats. The age of the mice at the beginning of the experiment was approximately 2 to 3 months.

The fractions were at first used undiluted, but as this resulted in a rather high initial mortality they were later mixed with a small amount of acetone, sufficient for solution of the bulk. Only fraction 1, which was liquid, was used undiluted throughout.

The mice painted with fraction 2 showed such a high mortality that a second series, consisting of 19 mice, was started about 7 months after the beginning of the experiment. By using an acetone solution of the fraction the mortality was greatly reduced in this second series, 17 mice out of 19 surviving for more than 18 months.

The painting of fraction 3 had to be interrupted after 5½ months owing to lack of material and could only be started again after an interval of 3½ months. Fraction 2 also became exhausted towards the end of the 19th month after the beginning of the second experiment. The survivors of this series were killed about 6 weeks later. Otherwise painting continued throughout the life of the animals, which were allowed to die of natural causes.

The length of life of the animals in each group is shown in Fig. 1. It will be seen that, except in the first fraction 2 series, a sufficient number of mice in each group reached a high age, so that there was a reasonable chance of even a weakly carcinogenic activity being discovered.

RESULTS

Local effects.—All fractions were irritant and caused epilation at the site of application, as was also noted by Valade (17). The loss of hair, which started after about 3 weeks, was at first only temporary and normal fur grew again to be followed by a second and more permanent baldness. The skin later showed eczematous changes in many cases.

One of the mice painted with fraction 1 developed a small soft papilloma at the edge of the bald area caused by the treatment. It appeared after 22 months and regressed spontaneously a month later. When the mouse died, 24 months after the beginning of the experiment, no sign of it remained.

In no other case has any abnormal growth been observed at the site of application.

Remote and general effects.—As pointed out already most fractions caused a fairly high initial mortality. In the case of fraction 2 this could be controlled by using an acetone solution instead of the undiluted fraction when the repetition of the experiment was started. Once the initial ill effects had been overcome, the mice did not seem to suffer from the treatment and appeared to be in good health.

An autopsy was carried out whenever possible. In most cases, especially in young animals, no gross pathological changes could be detected and it was assumed that intercurrent infections had caused death. In other cases, especially in the older animals, diagnosis was possible, but no neoplastic changes were observed.

Neoplastic changes.—Excepting in the mice painted with fraction 2 (second batch), only a few examples of neoplastic changes were observed. These were: one case of leukemia and one of hepatoma among the mice painted with fraction 1, one mucous cyst of the abdominal wall in the group painted with fraction 3, and one cervical cyst containing heterotopic tissues in the group painted with fraction 4. Nothing of inter-

est was observed in the group of controls. In view of the isolated and sporadic occurrence of these tumors no conclusions as to their possible connection with the experiment can be drawn.

Observations of some consequence were made only in the group of mice painted with fraction 2 (second batch). Of 17 animals surviving for 18 months or more, 8 had single or multiple adenomas of the lung. These were round nodules of solid white tissue situated beneath the pleura. They measured 1 to 3 mm. in diameter; one tumor measured 5 mm. One mouse had 3 separate tumors and two had 2. Histologically the nodules consisted of strands of well differentiated columnar and cuboidal epithelium forming irregular acini. The cells were for the most part regular in size and shape though some showed nuclear hypertrophy and hyperchromatism. The tumors were often necrotic in the center, they were not encapsulated, and though some were less well differentiated than others definite signs of malignancy were absent.

One of the mice bearing an adenoma of the lung had in addition a liver cell carcinoma; another animal, whose lungs were normal, had a lymphosarcoma situated beneath the right lobe of the liver.

Feeding Experiments

The effect of smoked food as a dietary item was tested in a series of feeding experiments on rats. In the first experiment a group of 20 black-hooded white rats were fed well smoked kippers only for 3 days weekly, while a control group of 20 rats received salted herrings instead. For the rest of the week the rats were kept on our usual laboratory diet of oats and dog-meal with a weekly supplement of cod liver oil and marmite. In the second year of the experiment difficulties of supply led to some irregularities and interruptions.

The animals had an average weight of 70 gm. at the beginning of the experiment. Both groups showed normal development and growth and good general

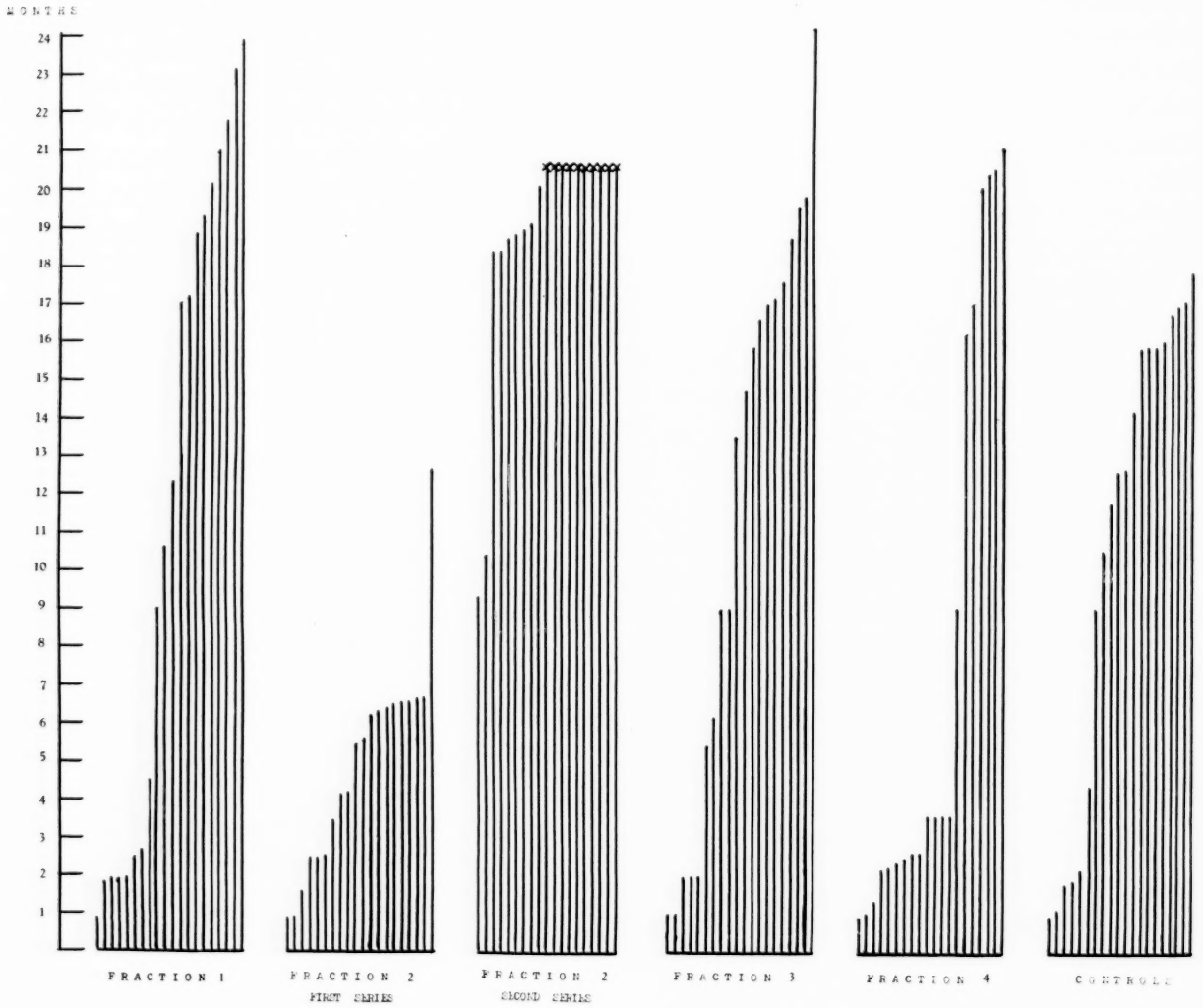

Fig. 1.—Survival of mice in months. x = killed.

health. The average weight after 12 months was 300 gm. After 20 months all the rats had died and in no case was any neoplasm found. No ulcerations or other possible precancerous changes were found in their stomachs or intestines.

In a second experiment 20 rats received a diet of heavily smoked pork. This experiment, which suffered still more from irregularities of supply, was continued for 12 months. The last rat died after 18 months. No neoplasm developed in this series either.

DISCUSSION

The essential result of our experiments is the absence of local tumor formation. Only one case has been observed where a typical wart developed after 22 months and this spontaneously regressed a month later. As the application was continued for long periods, 24 months in some cases, it may be concluded that wood smoke does not produce epithelial tumors of the skin when painted on mice.

This is hardly surprising in view of the low temperature (400–450° C.) of the combustion, a point which is also stressed by Valade (17). Even coal tars produced at about 450° are not carcinogenic (9), or are only weakly so (6), while tars with high activity, whether synthetic or from the distillation of coal or of vegetable or animal matter, are prepared at temperatures of 700–900°. It is in accordance with this that the chemical analysis of wood smoke (13) did not reveal the presence of hydrocarbons and that none of the substances which were actually found has so far been shown to possess carcinogenic activity.

The slight carcinogenic effect of tobacco smoke condensates is probably also due to the considerably higher temperature (520–730°) which is reached in the pipe during puffs (5).

The question now arises whether the skin of mice is the most susceptible test object. Work with pure carcinogenic compounds has shown that some have a remarkable affinity for organs remote from the site of application, while others act locally but are much more effective when injected subcutaneously than when applied to the skin or *vice versa* (3). Other substances, *e.g.* tobacco tars (5), produce tumors much more readily when painted on the ears of rabbits than on the skin of mice. By way of contrast, the mouse responds more readily to the cutaneous application of pure carcinogenic hydrocarbons than the rabbit (16).

In this connection our observation of pulmonary adenomas in 47 per cent of the mice which had been painted with fraction 2 for 18 months must be regarded as a significant result, although it would be desirable to repeat the experiment with a pure strain, in which the spontaneous incidence of lung tumors at a given age is known. We hope to be able to investigate this point in the future. In our experience spontaneous pulmonary tumors are rare among our stock mice, even at an advanced age. The incidence among 147,132 mice of the Slye stock, dying of natural causes, is reported to be 2 per cent (18). The spontaneous occurrence of multiple lung adenomas must be rarer still; we have never seen a single case in our untreated stock mice. It is of course well known that pulmonary adenomas are frequently induced by the application of carcinogenic agents, especially in strains with a tendency to the spontaneous development of these tumors (1), but also in resistant strains (2). Sometimes pulmonary adenomas develop before any changes are noticeable at the site of application; these may even be missing entirely. It appears therefore that, at least in these cases, the lungs are a more sensitive test object than the skin. It is possible, and indeed probable, that ours is a case in point, and we therefore arrive at the tentative conclusion that fraction 2 possesses a very weak carcinogenic potency. It is perhaps more than a coincidence that the only other instances of true malignancy were observed in the same series; *i.e.*, a case of lymphosarcoma and a case of liver carcinoma.

The feeding experiments on rats gave an entirely negative result. It seems, however, that rats are not very susceptible to the induction of gastric cancer by this type of experiment as even strongly carcinogenic hydrocarbons, when fed to rats, had no effect (4, 11).

The great variation in cancer susceptibility of particular tissues among different species makes it very difficult to apply the results of animal experiments to human pathology. Thus a carcinogen might possess a specific affinity for the mucous membranes of the stomach. In a species where the absence or rarity of spontaneous gastric cancer indicates a low susceptibility of this organ (7) it might be harmless, especially if of low potency, and yet it might be of importance in the etiology of human gastric cancer. It is with these reservations in mind that we report our experiments which, in spite of the high concentrations used and the prolonged application, have failed to reveal more than an extremely weak carcinogenic activity.

SUMMARY

Four groups of 20 mice were painted twice a week for a period up to 24 months with 4 fractions of wood smoke condensates, prepared under conditions closely resembling those employed in the food-smoking industry. At the site of application only one papilloma was observed, which appeared after 22 months and spontaneously regressed a month later. On the other hand, single and multiple pulmonary adenomas were found in 8 of 17 mice painted for 18 months or more with

the tar fraction which had settled out from the "pyroligneous liquor."

In another series of experiments rats were fed smoked fish or meat 3 times weekly for periods up to 20 months. No neoplasms developed.

We wish to express our thanks to Dr. G. S. Whitby, Director of the Chemical Research Laboratory, Teddington, for preparing and supplying the smoke condensates; to Professor A. F. Bernard Shaw and Dr. J. G. Thompson, Department of Pathology, University of Durham, for histological examinations; and to Professor Sir Edward Mellanby and Professor E. L. Kennaway for helpful criticism.

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The Incidence of Primary Lung Tumors in Mice with Induced Sarcomas*†‡

Charles E. Dunlap, M.D., and Shields Warren, M.D.

(From the Harvard Cancer Commission and the Department of Pathology, Harvard Medical School, Boston, Mass.)

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It is well recognized that different species of animals and different strains within the same species show definite statistical differences in susceptibility to spontaneous and to induced tumors. The present study is concerned not so much with species or strain differences as with the individual differences in tumor susceptibility among mice of a single highly inbred strain. Genetic variation is here reduced to a minimum and other factors may be observed against a less complicated background.

When a large group of mice is exposed to the action of a carcinogenic agent something less than 100 per cent of the animals develop tumors. Some may die of other causes before the average time necessary for tumor induction but some survive for long periods beyond the time when most of their fellows have died of cancer.

Failure to develop a tumor under these conditions might depend on some specific biological resistance on the part of the refractory mice, such, for example, as a particularly effective mechanism for inactivating and disposing of the carcinogenic agent or a lack of capacity of the tissue cells to undergo readily malignant change. On the other hand, failure might depend on some phenomenon of chance such as a fortuitous failure of the carcinogenic agent or its derivatives to come into effective contact with a suitable group of cells in the proper concentration and at the right time to induce malignant change.

The same reasoning may be applied to the irregular distribution of spontaneous tumors in a population of mice or of men. The natural scatter may reflect basic differences in individual tumor susceptibility but it might also represent the chance operation of precipitating factors in a group of equally susceptible individuals.

Mice exposed to the action of a carcinogenic agent generally develop tumors at the site of application of the agent. Certain mice so exposed will also develop growths at a distance, particularly in the lungs. The local tumors, produced at the site of subcutaneous injection, are virtually all sarcomas. The characteristic cells are spindle-shaped and grow in a diffuse disorganized fashion with little stroma. Tumor giant cells are common and mitotic figures are usually abundant. The tumors show active local invasion of muscle, fascia, and skin but metastases are uncommon and when present rarely extend beyond the regional lymph nodes. The majority show collagen between the tumor cells and are best classed as fibrosarcomas although a considerable number of rhabdomyosarcomas and neurofibrosarcomas are also found. Growths of epithelial origin almost never arise at the site of subcutaneous injection except when ulceration occurs and the carcinogenic agent is able to reach the skin surface.

The lung tumors, on the other hand, are adenomas and adenocarcinomas of characteristic alveolar structure. Most of them are subpleural but some arise in the deeper parts of the lung often adjacent to small bronchi. Growth appears to be expansile in most cases with compression of adjacent lung tissue. Mitotic figures are rare and true invasive growth or metastases are exceptional. Some of the larger tumors become papillary and may extend into the lumens of the bronchi, but the more common structure is alveolar with a single layer of cuboidal cells covering the surfaces of a delicately branching, reticulated fibrous stroma. The architecture often suggests an attempt to reproduce pulmonary alveoli and several authors, including Grady and Stewart (2), believe that the tumors originate from alveolar lining cells. Spontaneous growths of identical appearance are found quite frequently in old mice of several albino strains. The spontaneous adenomas are generally solitary, while as many as 100 distinct tumors may be found in the lungs of mice injected with carcinogenic agents. The pulmonary neoplasms may occur with or without tumors elsewhere in the body and may safely be considered as primary in the lung rather than metastatic.

It appears, therefore, that carcinogenic agents may

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‡ The compounds used in these experiments were selected and synthesized by Professor L. F. Fieser and his associates of the Department of Chemistry, Harvard University.

induce 2 easily distinguishable types of tumor in different parts of the body of the same mouse. We thus have the same biological substrate exposed to the risk of developing 2 independent growths. If all mice in an exposed group are equally susceptible in all respects to tumor induction then the pulmonary neoplasms and the sarcomas should be distributed in chance fashion throughout the group. But if there are true individual differences in general susceptibility we should expect to find both the lung tumors and the sarcomas concentrated in the more susceptible mice.

Our data come from autopsies on pure strain C3H and Swiss mice obtained from the Roscoe B. Jackson Laboratory at Bar Harbor, Maine. Only male mice were used, since the females of both strains may develop spontaneous breast tumors. They were kept in metal cages in groups of 5 and were fed a uniform diet of Purina dog chow and water. At the age of 3 to 5 months all mice were injected subcutaneously or intraperitoneally with one of 55 different synthetic hydrocarbon derivatives prepared by Professor Louis F. Fieser and his associates at Harvard University. The dose, with few exceptions, was 2 mgm. per mouse dissolved in tricaprylin. Since the initial purpose of these injections was to assay the carcinogenic potency of new compounds, considerable care was taken to administer accurately measured doses by a standardized technic. All animals dying at any time after injection were autopsied and all tissues macroscopically suggestive of tumor were examined histologically. We have thus accumulated 2,729 autopsies on mice dying at all ages from 3 months to 2½ years.

Autopsies were performed on 1,705 mice of the C3H strain. This strain has a high incidence of breast tumors in females but growths in the males are uncommon. Strong (6), who originated the strain, observed no tumors except in the breast during 15 years of continuous observation; Andervont (1), however, reported an incidence of spontaneous lung tumors of over 4 per cent in C3H males between 8 and 19 months of age and a much higher incidence in mice injected with carcinogenic agents. In our experience only 4 adenomas of the lung were discovered in 1,705 autopsies, although 281 subcutaneous sarcomas were induced. We also failed to find the high incidence of primary hepatomas described by Andervont (1). However, we do not find these apparent discrepancies disconcerting. The work of the last few years has disclosed many environmental and dietary factors which exercise major influences on the occurrence of animal tumors. Unavoidable differences in the feeding, housing, and care of animals in various laboratories might easily explain the observed differences in incidence. It seems necessary for each investigator to determine for himself the incidence of tumors under the conditions peculiar to his own colony. The data of others give valuable indications of what may be expected but seldom represent the result of strictly comparable experiments. Under the conditions of our work subcutaneous sarcomas were readily induced in C3H mice but pulmonary adenomas and hepatomas were exceedingly rare.

The remainder of this paper will deal exclusively with results obtained on mice of the Swiss strain. This strain was introduced by Lynch in 1926, and in her hands (4) the incidence of spontaneous lung tumors in 684 animals of all ages was 43.9 per cent. In our series of 1,024 autopsies, including mice injected with active carcinogenic agents, only 99 were found to have lung tumors, an over-all incidence of less than 10 per cent. The median time of death was 200 days after injection, which corresponds to an age of approximately 9 months.

Of these autopsies, 586 were performed on Swiss mice exposed to 33 compounds which uniformly failed to produce neoplasms at the site of injection. In this group 31 mice were found to have primary lung tumors, an incidence of 5.3 per cent. The average time between injection and the discovery of the growths was 483 days.

TABLE I: PRIMARY LUNG TUMORS IN SWISS MICE

	Number of autopsies	Incidence of lung tumors, per cent	Average induction time, days
All inactive compounds	586	5.3	483
All active compounds	438	15.5	294
Four highly active compounds	80	39.0	197

Autopsies were also performed on 438 mice exposed to 22 "active" compounds, each of which had produced one or more tumors at the site of injection. In this group 68, or 15.5 per cent, had primary lung tumors and the average elapsed time was only 294 days.

Many of the compounds classed as "active" were extremely weak carcinogenic agents. Therefore the mice exposed to 4 highly active agents, 20- and 22-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene are considered separately. In this group 80 autopsies revealed 31 lung tumors, or an incidence of 39 per cent. The average induction time was 197 days.

These data, combined in Table I, demonstrate that the subcutaneous or intraperitoneal injection of carcinogenic agents into Swiss mice increases the incidence and hastens the appearance of primary lung tumors. Numerous investigators have reported similar results (5).

The next question to consider is whether the distribution of lung tumors within the exposed mouse population bears any relationship to the distribution

of tumors induced at the site of injection of the carcinogenic agent.

Considering first the 438 mice injected with "active" compounds, we found that 82 developed growths at the site of injection and died at a median time of 125 days after injection. Of these 33, or 40.2 per cent, also had primary lung tumors. After injection with these same compounds, 356 mice failed to develop neoplasms at the site of injection and died at a median time of 170 days after injection. Of these only 35, or 9.8 per cent, developed lung tumors. Thus in the combined groups of mice injected with 22 different carcinogenic agents, those mice which developed growths at the site of injection showed an incidence of lung tumors 4 times greater than their fellows even though they died at an earlier median age.

Of the group of mice injected with the 4 active compounds, 20- and 22-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene, 36 developed tumors at the site of injection and 25 of these, or 69 per cent, also had lung tumors; 44 failed to develop growths at the site of injection and of these only 6, or 12.5 per cent, had lung tumors. Thus, in this group, the mice with neoplasms at the site of injection had over 5 times greater risk of lung tumors than their fellows. Even if we exclude all mice that died without local tumors during the first 6 months of the experiment, and consider only those that survived far beyond the average tumor induction time, we find that these old mice have only half the incidence of lung growths found in the younger mice who died with tumors at the site of injection. These data are collected in Table II.

TABLE II: MULTIPLE TUMORS

	Incidence of tumors at site of injection, per cent	Incidence of all primary lung tumors, per cent	Incidence of primary lung tumors in mice with tumors at the site of injection, per cent	Incidence of primary lung tumors in mice without tumors at the site of injection, per cent
All carcinogenic compounds	18.7	15.5	40.2	9.8
Four highly active compounds	47.0	39.0	69.0	12.5

It is a somewhat questionable procedure, statistically, to combine groups of mice injected with different compounds and treat the whole as a single experiment. No group of mice numbering more than 25 was exposed to a single compound and thus the individual experiments are not statistically useful. However, all the experiments with single compounds which produced more than 5 tumors at the site of injection showed a preponderance of lung tumors in those mice which also developed subcutaneous growths. We therefore feel justified in concluding that in combined averages of groups of pure strain Swiss mice injected in the same fashion with identical amounts of the same carcinogenic agents, those mice which develop tumors at the site of injection have a higher incidence of primary lung tumors than their fellows.

This appears to indicate that the distribution of these 2 types of tumor in an exposed mouse population is not a matter of chance alone, but that certain mice within an inbred strain have an individual susceptibility or resistance to both subcutaneous sarcomas and primary lung tumors.

We are not prepared to explain these individual differences in susceptibility on a genetic basis. The state of nutrition or general health of an animal might well be the important factor. We have found, as have others, that mice which are poorly nourished or diseased respond poorly to attempts to induce tumors. At any rate, the data indicate that under the conditions of this experiment the susceptibilities of mice to 2 different types of induced tumor go hand in hand.

A somewhat comparable state of affairs has been demonstrated among human beings by Warren and Gates (7) and by Lund (3). Persons who have had one malignant tumor suffer a greater risk of developing a second, unrelated malignant tumor than would normally be expected in a group of like age and sex.

SUMMARY

1. C3H mice are more resistant than Swiss mice to the induction of primary lung tumors.

2. In Swiss mice, carcinogenic agents increase the incidence and hasten the appearance of lung tumors.

3. The incidence of primary lung tumors among Swiss mice is several times higher in those mice developing neoplasms at the site of injection of a carcinogenic agent than it is in mice treated identically but failing to develop such growths.

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The Inhibition of Sulfhydryl-Containing Enzymes by Split Products of p-Dimethylaminoazobenzene*

V. R. Potter, Ph.D.

(From the McArdle Memorial Laboratory, the Medical School, University of Wisconsin, Madison, Wis.)

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Following the demonstration (7) that the coenzyme I content of rat liver tumors is considerably lowered, Kensler, Dexter, and Rhoads (5) were led to investigate the effect of *p*-dimethylaminoazobenzene in a coenzyme I system *in vitro,* as well as to study the inhibitory action of certain compounds which were shown to be metabolically derived from this dye (18). They showed that the toxicity of the various split products was directly correlated with the carcinogenicity of the parent compounds, the stability of the free radical, and the ease of oxidation of the reduced compound. These reports seemed to us to have a direct bearing on the problem of the mechanism of carcinogenesis, and we at once took up the study of the effect of the split products of *p*-dimethylaminoazobenzene upon the succinoxidase system, which we had developed for just such a purpose (11, 13). That this enzyme system is inhibited by the split products had been shown in preliminary experiments by Kensler (private communication) and by Ball (2), who reported that *p*-phenylenediamine was toxic to succinic dehydrogenase.

It is highly desirable that the studies on yeast zymase described by Kensler be extended to other enzyme systems so that the nature of the inhibition may be better understood, and so that the mechanism of carcinogenesis will not be prematurely ascribed to the inhibition of any one particular enzyme. After a number of experiments with the succinoxidase system of rat liver (12) we realized that a still simpler enzyme system would facilitate the study. We therefore turned to urease, a plant enzyme which is considered to require an intact SH group for its action (4), and which consists of a single biocatalyst, in contrast to the succinoxidase and zymase systems which require the coordinated functioning of at least 3 and 10 biocatalysts, respectively. The present report contains the data obtained with the urease system, which was used in this instance merely as a tool, in order to help clarify the results with the succinoxidase and zymase systems.

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EXPERIMENTAL

Test system.—The commercial jack bean urease preparation sold by the Arlington Chemical Company was used as the source of enzyme throughout the experiments. Since the enzyme catalyzes the reaction

$$NH_2—CO—NH_2 + H_2O \rightarrow 2\ NH_3 + CO_2$$

the activity of the enzyme may be measured on the basis of the rate of production of either ammonia or CO_2. We measured the evolution of CO_2 since this can be carried out conveniently with the usual Warburg apparatus, especially when the medium is acid enough to give a low α value for CO_2. Kubowitz and Haas (9) have used this technic for the measurement of urease activity. They pointed out that the activity at pH 5.66 is 3.5 times lower than at pH 7.0. We used an acetate buffer at pH 5.0 since that is the pH at which Michaelis, Schubert, and Granick (10) studied the free radicals of the type of Wurster's salts, which happen to be identical in many cases with the split products of *p*-dimethylaminoazobenzene and related compounds, and which are more stable in this pH range than at pH 7.0.

The amount of enzyme was chosen so as to give a manometric displacement of just under 300 mm. over a period of 1 hour. The rate is much greater during the first 10 minutes than in subsequent periods because traces of heavy metals in the system have not taken effect in the earlier period. The activity is calculated on the basis of the average rate during the last four 10-minute periods of the hour. This rate is constant in the controls, which are included in every run. The rate is proportional to $E\text{-}E_m$, where E is the amount of enzyme added and E_m is the amount of enzyme which is inactivated by the heavy metals in the system. The value for E_m appears to be proportional to the heavy metals present and independent of E, so that when CO_2 per hour is plotted against E, a straight line results which intersects the E scale at E_m. The amount of enzyme used was 200 γ but with another batch of acetate buffer the value for E_m was so much smaller that only 50 γ of enzyme were required. Since

dilute solutions of the enzyme rapidly lose activity, the solutions are prepared immediately before use.

The optimum buffer molarity was determined as shown in Table I and the amount of substrate required to saturate the enzyme throughout the experimental period was determined as shown in Table II, which also illustrates the lability of the enzyme preparation. The final test system is described at the top of Table III.

Compounds tested.—Cysteine was from Hoffmann-LaRoche. Hydroquinone was sublimed from the Merck product. The 2-methyl-N^4, N^4-dimethyl-1,4-phenylenediamine (meta compound) and 2-methyl-N^1, N^1-dimethyl-1,4-phenylenediamine (ortho compound) were kindly supplied by C. J. Kensler. Other compounds were standard Eastman products, as received. The compounds were dissolved at a concentration of 0.01 M in acetate buffer pH 5.0 of the same molarity. Further dilutions of the compounds were then made with the same buffer.

Table I: Effect of Buffer Molarity on Urease Activity

Each flask contained 1.0 M acetate buffer pH 5.0 as shown, plus 0.3 ml. of 0.5 M urea, 0.4 mgm. of urease in 0.2 ml. water, and water to make 3.0 ml.

1.0 M buffer, ml.	Final buffer molarity	CO_2 per 10 minutes
0.5	0.167	29.8
1.0	0.333	34.1
1.5	0.500	34.4
2.0	0.667	31.1

Table II: Effect of Substrate Concentration on Urease Activity

Each flask contained 0.5 M urea as shown, plus 1.0 ml. of acetate buffer pH 5.0, 0.4 mgm. of urease in 0.2 ml. water, and water to make 3.0 ml.

0.5 M urea, ml.	Final urea molarity	CO_2 per 10 minutes
Experiment A		
0.1	0.017	37.6
0.2	0.033	50.9
Experiment B *		
0.2	0.033	32.0
0.3	0.050	34.4
0.4	0.067	34.7

* Experiment B was run 5 hours after experiment A with the same enzyme solution and illustrates the lability of the enzyme.

RESULTS

Inhibitors alone.—Kensler and his associates (5) reported that the various split products had to be oxidized in order to be toxic. The results in Table III support this conclusion, since quinone is shown to be toxic at a concentration at which hydroquinone alone is completely nontoxic. Quastel (14) had previously reached the same conclusion with the urease system although his results are not as striking, since he reported 98 per cent inhibition by quinone and 64 per cent inhibition by hydroquinone when each was at a concentration of 1:2,500,000. Quastel studied the system at a pH of 7.4, at which hydroquinone is much more autoxidizable than at pH 5.0. Table III shows that in order to produce comparable inhibition with quinone and hydroquinone, the latter had to be increased to a concentration about 300 times that of the quinone. An autoxidation of only 0.3 per cent would account for the result. It is also shown in this table that the inhibition of urease by quinone is not instantaneous but proceeds steadily for about an hour. However it is more convenient to leave the inhibitor in contact with the urease arbitrarily for a period of 20 minutes, and this was the routine procedure. The split products were tested at various concentrations and the results at M/1,000 are reported in Table III. In general the results parallel those of Kensler, with the toxicity roughly parallel to the stability of the free radical and the oxidizability of the inhibitors. These latter functions parallel each other in the case of the diamines and therefore the data correlate with either function. In the case of hydroquinone the free radical concentration must be very small, yet the compound is readily autoxidizable. It is just as toxic as the diamines which have stable free radicals and which are readily oxidized. The results with hydroquinone

Table III: Inhibition of Urease by Quinone, Hydroquinone, and Related Diamines

Each flask contained 0.9 ml. of 1.0 M acetate buffer pH 5.0, 0.4 ml. of 0.5 M urea dissolved in buffer (in side arm), 0.2 mgm. of urease in 0.2 ml. water, plus inhibitor and water to make 3.0 ml.

Inhibitor	Final molarity	Per cent inhibition
Quinone	2×10^{-6}	85 *
Quinone	2×10^{-6}	73 *
Quinone	2×10^{-6}	57
Quinone	3.3×10^{-6}	87
Quinone	1×10^{-5}	97
Hydroquinone	2×10^{-6}	0
Hydroquinone	3.3×10^{-4}	48
Hydroquinone	1×10^{-3}	75
p-Aminophenol †	1×10^{-3}	84
p-Phenylenediamine	1×10^{-3}	45
N-Methyl-*p*-phenylenediamine	1×10^{-3}	66
N,N-Dimethyl-*p*-phenylenediamine	1×10^{-3}	64
2-Methyl-N^4,N^4-dimethyl-1,4-phenylenediamine (meta)	1×10^{-3}	75
2-Methyl-N^1,N^1-dimethyl-1,4-phenylenediamine (ortho)	1×10^{-3}	0

* Enzyme in contact with inhibitor for 50 and 30 minutes respectively before substrate was added from side arm. In all other cases, the time was 20 minutes ± 3 minutes.

† Difficulty in preparing solution probably gave greater oxidation of the inhibitor.

and quinone deviate sharply from the results obtained by Kensler and his associates with these compounds in the zymase system. With urease hydroquinone has about the same toxicity as the diamines, and quinone is about 300 times more toxic. In the zymase system it was reported that quinone was half as toxic as N,N-dimethyl-*p*-phenylenediamine, while hydroquinone was completely nontoxic. The fact that quinone, alone or with the other end products of the diamine oxidation, was less toxic than the diamine led to the elimination of the fully oxidized products as the toxic factors in the zymase system, but such a conclusion is plainly not permissible here.

TABLE IV: EFFECT OF CYSTEINE ON INHIBITION OF UREASE BY QUINONE, HYDROQUINONE, AND RELATED DIAMINES

Basic reaction mixture as in Table III; cysteine was added in certain cases which are indicated

Inhibitor	Cysteine	Q_{CO_2}
None	None	1,115
Hydroquinone, 1×10^{-3} M	None	255
None	1×10^{-3} M	1,563
Hydroquinone, 1×10^{-3} M	1×10^{-3} M	1,545
Quinone, 3.3×10^{-6} M	None	150
Quinone, 3.3×10^{-6} M	1×10^{-3} M	1,551
Quinone, 1×10^{-4} M	1×10^{-3} M	1,500
Quinone, 1×10^{-5} M	2×10^{-5} M	30
None	3.3×10^{-6} M	1,119
Quinone, 3.3×10^{-6} M	None	222
	1×10^{-3} M at 40 min.	222
None	None	1,290
p-Phenylenediamine, 1×10^{-3} M	None	630
None	1×10^{-3} M	1,860
p-Phenylenediamine, 1×10^{-3} M	1×10^{-3} M	1,842
p-Phenylenediamine, 1×10^{-3} M	None	561
	1×10^{-3} M at 40 min.	658
N,N-Dimethyl-*p*-phenylenediamine, 1×10^{-3} M	None	378
N,N-Dimethyl-*p*-phenylenediamine, 1×10^{-3} M	1×10^{-3} M	1,815
N,N-Dimethyl-*p*-phenylenediamine, 1×10^{-3} M	None	387
	1×10^{-3} M at 40 min.	369

Inhibitors with cysteine.—Additional grounds for eliminating the fully oxidized products as toxic factors in the zymase system were based on the observation that the toxicity of quinone and *p*-phenylenediamine could be prevented with cysteine, while that of N,N-dimethyl-*p*-phenylenediamine could not. In definite contrast to this observation are the results in Table IV, which show that in the urease system, cysteine effectively prevented the inhibition by all the compounds. On the other hand, the toxicity was not reversed in any case by the action of cysteine (Table IV). Thus the results obtained with quinone are no different from the results obtained with the diamines in this regard, and again the fully oxidized split products are not eliminated as the toxic agents. The activating effect of cysteine is considered to depend on the fact that traces of heavy metals are present in the system.

Inhibitors with bromine.—One of the compounds with an extremely unstable free radical is the 2-methyl-N^1,N^1-dimethyl-*p*-phenylenediamine. This may be referred to as the ortho compound in contrast to the 2-methyl-N^4,N^4-dimethyl-*p*-phenylenediamine in which the methyl on the ring is meta to the dimethyl amino group. The ortho compound has an unstable free radical, is not autoxidizable, and is nontoxic. The meta compound is just the opposite in all 3 categories. On the basis of these facts it is not possible to decide whether the toxicity is correlated with the stability of the free radical or with the ease of oxidation. As a matter of fact one might expect a stable free radical to be less reactive than an unstable one, since stability and reactivity are expressions covering what is possibly

TABLE V: INHIBITION OF UREASE BY INHIBITORS WHICH ARE PARTIALLY OXIDIZED WITH BROMINE

Basic reaction mixture as in Table III; in the cases indicated the inhibitors were treated with bromine prior to addition to the flasks

Inhibitor	Bromine	Per cent inhibition
None	1×10^{-6} N	22
None	1×10^{-5} N	99
Hydroquinone, 1×10^{-4} M	—	32
Hydroquinone, 1×10^{-4} M	1×10^{-5} N	91
Quinone, 5×10^{-6} M	—	96
p-Phenylenediamine, 1×10^{-4} M	—	5
p-Phenylenediamine, 1×10^{-4} M	1×10^{-5} N	11
N,N-Dimethyl-*p*-phenylenediamine, 1×10^{-4} M	—	26
N,N-Dimethyl-*p*-phenylenediamine, 1×10^{-4} M	1×10^{-5} N	24
2-Methyl-N^4,N^4-dimethyl-*p*-phenylenediamine 1×10^{-4} M (meta)	—	48
2-Methyl-N^4,N^4-dimethyl-*p*-phenylenediamine 1×10^{-4} M (meta)	1×10^{-5} N	52
2-Methyl-N^1,N^1-dimethyl-*p*-phenylenediamine 1×10^{-4} M (ortho)	—	0
2-Methyl-N^1,N^1-dimethyl-*p*-phenylenediamine 1×10^{-4} M (ortho)	1×10^{-5} N	35

the same property. An unstable free radical may disappear by several reactions all of which would lead to the impression of instability. It may dimerize, disintegrate, dismutate, or react with its environment. However the fundamental cause for instability would remain the same, namely an absence of a coplanar atomic arrangement, according to Michaelis and his group (10). We felt that bromine oxidation might enable us to decide whether the lack of toxicity in the ortho compound is due to the instability of the free radical or to the fact that it simply is not oxidized in the urease and zymase systems. Michaelis has demonstrated that all these compounds may be potentiometrically titrated with bromine, showing that it is an effective oxidant for them. When the ortho compound was partially oxidized with bromine it was no longer nontoxic but showed an appreciable inhibition (Table V), which could scarcely have been caused

by the free radical since the color of the free radical was not detectable in the Warburg flask, in contrast to the strong pink color from the free radical in the case of the meta compound. It thus appeared that the lack of toxicity in the case of the ortho compound results from its failure to be oxidized rather than to the instability of the free radical.

DISCUSSION

Although urease is a plant enzyme and its function is hydrolytic in nature, its dependence upon intact SH groups and its simplicity make it well suited for the present study. The difference in results as compared with the zymase system may be caused by fundamental differences in the mechanism of toxicity or by extraneous differences between the 2 systems. It seems significant in this connection that the findings (12) with the succinoxidase system of rat liver are perfectly analogous in every respect with the urease results and lead to the same conclusions. These results are summarized in Table VI. The succinoxidase system differs notably from the urease system in that it includes cytochrome *c* and cytochrome oxidase (Fig. 1), which have the well known ability to oxidize hydroquinone and *p*-phenylenediamine with great vigor. In that system, bromine is not necessary in order to effect the oxidation of the ortho compound, provided the conditions are properly arranged. If succinate is omitted for 20 or 30 minutes, the cytochrome system oxidizes the ortho compound so vigorously that it is oxidized to just as great an extent as the meta compound (which is autoxidizable). When the succinate is then added from the side arm it is seen that the 2 compounds have inhibited the succinic dehydrogenase to the same extent. Yet the free radical stability of the ortho compound could not have been increased by the experimental conditions since it is a fundamental property associated with atomic arrangement. It might be suggested that the succinoxidase system simply differs fundamentally from the urease system with regard to its response to the ortho and meta compounds, but experiments with succinate added first refute this argument. Under these conditions the succinate plus succinic dehydrogenase partially reduce cytochrome *c* and cut down the catalytic

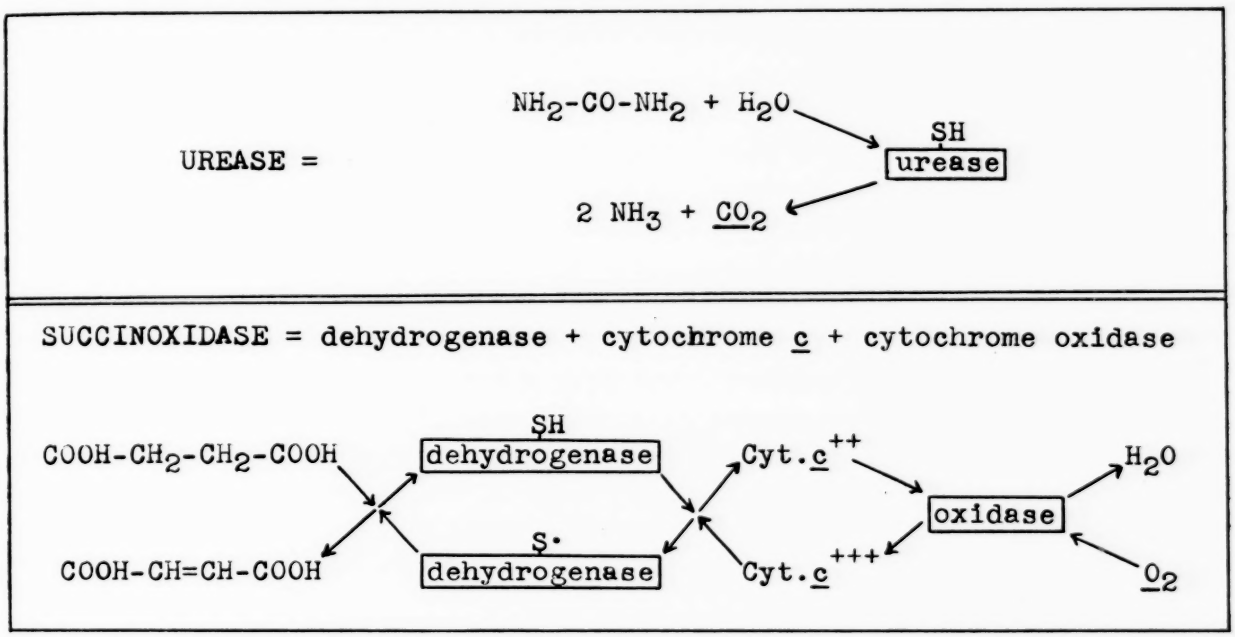

FIG. 1.—Schematic diagram of urease and succinoxidase systems indicating possible reaction mechanism in latter.

TABLE VI: INHIBITION OF THE SUCCINOXIDASE SYSTEM BY SPLIT PRODUCTS OF *p*-DIMETHYLAMINOAZOBENZENE AND RELATED COMPOUNDS

Data selected and condensed from (12)
Test system described elsewhere (11, 13)

Inhibitor	Per cent inhibition *	
	Succinate added before inhibitor †	Succinate added 40 min. after inhibitor ‡
p-Phenylenediamine	54	65
N-Methyl-*p*-phenylenediamine	65	65
N,N-Dimethyl-*p*-phenylenediamine	77	71
2-Methyl-N^4,N^4-dimethyl-*p*-phenylenediamine (meta)	26	72
2-Methyl-N^1,N^1-dimethyl-*p*-phenylenediamine (ortho)	0	68
Quinone	68	65
Hydroquinone	35	65

* At 30 to 40 minutes.
† Final molarity of inhibitor = M/30,000.
‡ Final molarity of inhibitor = M/100,000.

oxidation of the inhibitors by the cytochrome system to such an extent that the autoxidizable meta compound is oxidized to a much greater extent than the ortho compound, and therefore inhibits the dehydrogenase much more strongly than the latter. Thus the results are in complete harmony with those obtained with the urease system. It might therefore be suggested that the mechanism of inhibition may be the same in all 3 cases and that sulfhydryl-containing enzymes in general may be inhibited by the split products of *p*-dimethylaminoazobenzene. It remains to be seen which are the most sensitive and whether these are intimately connected with the mechanism of carcinogenesis.

The more obvious mechanisms of inhibition which might be suggested for quinone are:

1. $EnSH + C_6H_4O_2 \rightarrow (En—S— + C_6H_3(OH)_2—) \rightarrow C_6H_3(OH)_2—S—En$
2. $2\ EnSH + C_6H_4O_2 \rightarrow En—S—S—En + C_6H_4(OH)_2$(hydroquinone)

According to the first mechanism the reduced enzyme enters into chemical combination with the quinone. It appears that this reaction might be visualized as the oxidation of the enzyme to a free sulfhydryl radical with the concomitant reduction of the quinone to the semiquinone. The 2 free radicals could then combine to yield the addition product shown, which is a substituted hydroquinone. The over-all reaction has been demonstrated for R-SH by Snell and Weissberger (17). The inhibition might be expected to be irreversible. According to the second mechanism the quinone is simply reduced to hydroquinone by 2 molecules of En-SH, converting the enzyme to the S-S form. This reaction is also known to occur in the case of R-SH (17) and might be expected to be reversible.

We reject mechanism 2 in favor of mechanism 1 for 2 reasons. First, the inhibition appears to be irreversible (Table IV). Second, the sensitivity of the urease and succinoxidase systems to these inhibitors is about the same (Tables III, V, and VI). The second point requires some explanation. The data show that small quantities of hydroquinone are nontoxic and are not converted to quinone in any appreciable amount in the urease system, whereas hydroquinone is quickly converted to quinone in the succinoxidase system. Thus if mechanism 2 were the true mechanism of inhibition, hydroquinone would be reoxidized again and again in the succinoxidase system, but not in the urease system, and the inhibitors should be much more toxic in the succinoxidase system than in the urease system. According to mechanism 1, on the other hand, the inhibitors should inactivate the same amount of enzyme in each system. Since this is more in line with the facts we prefer to accept mechanism 1. It may be noted in passing that whereas in the urease system the inhibitor and enzyme must undergo mutual oxido-reduction to yield the free radicals which then combine, in the succinoxidase system it appears that both reactants can be reduced and oxidized by the other components of the system, and it seems not only possible but likely that the free radicals of both the enzyme and the inhibitor arise in the course of these other reactions (Fig. 1.). The reasons for this view will have to be discussed elsewhere (12).

The concept of irreversible inhibition of succinic dehydrogenase according to mechanism 1 is rather important from the standpoint of the cancer problem. If the enzyme is irreversibly inactivated the organism presumably has to replace the enzyme with a newly synthesized counterpart. The demonstration that the succinic dehydrogenase content of liver is lowered in flavin-deficient rats (1) indicates that this enzyme may be a flavoprotein and hence the cancer-preventing action of flavin and protein (8) in the case of animals fed *p*-dimethylaminoazobenzene may be partly explained. In this connection it may be noted that the liver is the site at which tumors are formed by feeding *p*-dimethylaminoazobenzene, and it is the only tissue in which succinic dehydrogenase is lowered by flavin deficiency (1). It may also be noted that the tumors formed by *p*-dimethylaminoazobenzene are deficient in succinic dehydrogenase (16). This is in harmony with the concept (3) that carcinogenic agents might be expected to inhibit enzyme systems which are essential to normal tissues but which are not essential to cancer tissue or to precancerous tissue.

Since the triose phosphate dehydrogenase which was inhibited in Kensler's zymase system is a key component in the glycolytic system in animal tissues it may be asked how the inhibition of this enzyme is compatible with the fact that the glycolysis of tumor tissue is high, in view of the above concept. The explanation may well be found in the fact that the glycolytic enzyme is almost certainly saturated with coenzyme I in normal liver *in vivo* and therefore protected against the inhibitors, since Kensler and his group (5) demonstrated that the coenzyme protects the triose phosphate dehydrogenase *in vitro*. We have shown (12) that succinate protects its dehydrogenase against the inhibitor also, but in this case it is almost certain that the dehydrogenase is *not* saturated with succinate *in vivo* (15), and hence would be open to action by the split products of *p*-dimethylaminoazobenzene. The fact that the split products must be oxidized in order to be toxic is highly significant, since the very enzyme which brings about the oxidation

is part of the system which is destroyed by the oxidized split product. Kensler and Rhoads (6) have suggested that cytochrome oxidase is destroyed by the split products of *p*-dimethylaminoazobenzene and we have shown that the tumors elicited by this compound are low in cytochrome *c* (3). At the sites where the inhibitors act *in vivo* the destruction of the succinoxidase system with relatively less action on the glycolytic mechanism will result in a situation in which the oxidation of the split products becomes less effective as glycolysis becomes more predominant. It would be premature to postulate a mechanism of carcinogenesis upon the few facts which are available at this time, but with the little information which is at hand one can begin to see the mechanism of a shift from an aerobic type of metabolism over to a glycolytic type. One can but speculate whether such a shift necessitates the abandoning of specialized functional energy requirements and a reversion to a more primitive type of cell.

SUMMARY

1. Split products of *p*-dimethylaminoazobenzene are shown to be highly toxic to a simple sulfhydryl-containing enzyme system (urease).

2. The split products were apparently nontoxic in the reduced state, *i.e.* as diamines, and became toxic only when oxidized.

3. The toxicity was correlated with both the autoxidizability of the diamines and their free radical stability, but even in the case of an unstable free radical, the compound was toxic if oxidized.

4. The inhibition was prevented but not reversed by cysteine.

5. The mechanism of inhibition is suggested to be a mutual oxido-reduction between the enzyme sulfhydryl and the oxidized split product followed by combination of the 2 end products.

6. The results are correlated with those obtained with 2 other sulfhydryl-containing enzymes and it is suggested that still other sulfhydryl-containing enzymes may be inhibited by the split products.

7. It is pointed out that the inhibition of succinic dehydrogenase by the split products of *p*-dimethylaminoazobenzene might be correlated with the effect of increasing the flavin and protein in the diet of rats fed this dye.

8. A possible mechanism for a shift from aerobic to glycolytic metabolism is discussed. It is suggested that whereas the succinic dehydrogenase might be expected to be inhibited *in vivo* as well as *in vitro*, the triose phosphate dehydrogenase might be inhibited *in vitro* but not *in vivo*.

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The Effect of Riboflavin on the Liver Changes Produced in Rats by p-Dimethylaminoazobenzene

William Antopol, M.D., and Klaus Unna, M.D.

(From the Merck Institute for Therapeutic Research, Rahway, and the Division of Laboratories, Newark Beth Israel Hospital, Newark, N. J.)

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As part of a study on the relation of nutrition to the production of tumors by carcinogenic agents, *p*-dimethylaminoazobenzene was used as a tumor-producing substance in rats maintained on synthetic diets to which individual vitamins were added in known amounts.

It was found by Kinosita (4) that, among other dyestuffs, *p*-dimethylaminoazobenzene produced cirrhosis and carcinoma when fed to rats. Rats maintained on a diet of polished rice were less resistant to the action of this compound than those maintained on unpolished rice. The influence of vitamins in these diets upon the activity of the dye was studied by other Japanese workers (6, 7), who found that the addition of yeast or liver to the rice diet prevented the formation of tumors. Sugiura and Rhoads (8) obtained analogous results when yeast (15 per cent), yeast extracts, and rice bran extracts were incorporated in the diet. The addition to the rice diets of thiamin, pyridoxine, nicotinic acid, or liver eluate, each substance alone or in combination with others, did not prevent the development of tumors (7).

The relationship between susceptibility to tumor development and the inclusion of vitamins of the B complex in the diet was further stressed by Rhoads and his collaborators, who demonstrated a notable increase in the riboflavin excretion of rats maintained on a diet of brown Texas rice supplemented with 0.06 per cent *p*-dimethylaminoazobenzene (2), and who showed that the livers of such rats had a diminished content of both riboflavin and coenzyme I (3). These investigators have also proved that the addition of both casein and riboflavin is necessary to prevent liver damage in rats maintained on the rice diet and that neither of these substances is effectual when administered alone (2). White (9) observed that rats kept on a low protein diet containing *p*-dimethylaminoazobenzene failed to gain weight normally, and that the addition of cystine or methionine prevented the retardation in growth caused by the carcinogen. György and his associates (1) reported that casein, or the combination of methionine, cystine, and choline, protected rats against pathological changes in the liver produced by various diets containing *p*-dimethylaminoazobenzene. Lewisohn's group (5), studying the prevention of growth of transplanted carcinoma in mice, found that the inhibiting effect of yeast was definitely improved by the addition of pantothenic acid or riboflavin.

As a result of the suggestion contained in the experiments of Rhoads, the effect of riboflavin and nicotinic acid on the tumor-producing action of *p*-dimethylaminoazobenzene when added to synthetic diets has been investigated in albino rats originating from a Wistar strain (total number, 78). The animals, housed in individual cages, were placed at 3 weeks of age on a riboflavin-free synthetic diet consisting of vitamin-free casein, 18 per cent; dextrose, 68 per cent; butter fat, 8 per cent; salt mixture U.S.P. XI No. 1, 4 per cent; and cod liver oil, 2 per cent. Of this diet 1 kg. was supplemented with 8 mgm. each of thiamin and pyridoxine, 50 mgm. of calcium pantothenate, and 1 gm. of choline chloride. *p*-Dimethylaminoazobenzene, in an amount of 0.02 per cent, was incorporated in the food either at the beginning of the dietary regimen (48 rats), or after a period of 3 weeks during which the rats maintained on the riboflavin-free diet received 10 γ of riboflavin 3 times a week (30 rats). Forty-four animals were given no further supplement of riboflavin from the beginning of the feeding of *p*-dimethylaminoazobenzene, whereas the other 34 received 10 mgm. of riboflavin 3 times a week by stomach tube. The riboflavin was administered either in an aqueous suspension or suspended in 0.5 cc. of gum acacia. Twenty-four animals received a supplement of nicotinic acid (50 mgm. 3 times weekly). Control groups of animals receiving no *p*-dimethylaminoazobenzene in the diet (total number, 30) were kept simultaneously over the same period of time. To another group, given *p*-dimethylaminoazobenzene in the diet, was administered gum acacia alone.

After 6 weeks on the riboflavin-free diet, the weights of the rats fed the dye became almost stationary at 60 to 80 gm. and generalized alopecia developed. No significant difference in weight or in external appearance was found between these rats and the control rats receiving no *p*-dimethylaminoazobenzene. The majority of the animals died within 6 to 8 weeks; only one-third survived for periods of more than 2 months. Rats that had the supplement of nicotinic acid succumbed somewhat earlier than those given no nicotinic acid.

All rats fed 10 mgm. of riboflavin 3 times a week continued to gain weight throughout the experiment and presented a normal healthy appearance. After 5 months these animals averaged 350 gm. in body weight and their weight curves did not differ significantly from those of control rats receiving no *p*-dimethyl-

aminoazobenzene. In a group of 4 rats, the riboflavin supplement was withdrawn after a period of 160 days. These rats responded with a gradual decline in weight and died within 5 to 6 months. All the animals were examined at intervals, as recorded in Table I, summarizing the liver changes noted.

The livers graded "o" showed no definite gross or histologic changes. This does not signify that minimal changes had not taken place. For example a rare nucleus might be larger or stain more deeply, or a cell might contain 2 nuclei, but on the whole the cell type was uniform and at most the lesions were only suggestive of those graded ±.

The livers graded ± showed minor changes in comparison with the higher grade. Some of these consisted of occasional cells with a more eosinophilic cytoplasm or larger nucleus. In most instances, the changes occurred in isolated cells rather than groups of cells. When they did occur in cell groups, the foci were considerably smaller and the changes less advanced than those found in livers graded 1+. A rare area of necrosis was found in some of the ± livers.

In the livers graded 1+ there were foci with dilated capillaries and smaller liver cells. The cytoplasm of these cells was usually pale, less eosinophilic, at times vacuolated, and the cells were often multinucleated. These foci were usually nearer the center of the lobule. The cells in the periportal region also were smaller, but in these the cytoplasm stained more densely; occasionally an increase in the number of bile ducts was seen. A rare hyaline thrombus was found in a capillary and occasionally a macrophage containing brown pigment was noted.

The livers graded 2+ showed varying degrees of disorganization. Extremely cellular areas, often nodular, were present, composed of smaller cells often running parallel to one another. These resembled the bile duct cells because of their scant cytoplasm, but bile duct formation by these cells was not striking. Some of the cells contained yellow pigment. Many of these cells were elongated, simulating fibroblasts and endothelial cells, and at times were stellate. These cellular areas appeared to encroach upon the adjacent liver tissue. Scattered cells with large nuclei, many of which had more than one nucleolus, were seen throughout. These were not in any fixed relation to the periportal fields but were irregularly situated, and had an abundant, often eosinophilic, cytoplasm; at

TABLE I: EFFECT OF THE FEEDING OF RIBOFLAVIN ON THE PRODUCTION OF LIVER CHANGES BY *p*-DIMETHYLAMINOAZOBENZENE IN RATS ON A PURIFIED DIET FREE FROM RIBOFLAVIN

No riboflavin				30 mgm. riboflavin per week			
Autopsy number	Final weight, grams	Time on diet, days	Liver involvement	Autopsy number	Final weight, grams	Time on diet, days	Liver involvement
2661 *	97	40	1+	1854	210	64	o
2662 *	68	40	1+	1929	265	80	o
2683 *	56	40	1+	1940 †	231	88	o
2684 *	63	40	1+	1976	224	92	o
2717 *	70	41	2+	3042 *	154	99	o
2718 *	76	43	2+	2051 †	223	108	o
2665 *	64	43	2+	2054	365	111	o
2719 *	74	44	2+	3098 *	207	120	±
2720 *	76	45	2+	3101 *	286	120	±
2666 *	86	53	±	3102 *	142	120	1+
2722 *	86	53	2+	2165 †	318	142	o
2725 *	92	56	1+	2256	362	177	o
1853	78	64	±	2257	388	177	o
1855 †	70	64	1+	2259	429	178	o
1920	86	78	2+	2260	327	178	o
1977	65	92	2+	3620 *	344	258	1+
1978	69	92	1+	3621 *	462	258	1+
1943	66	99	1+	2628	415	316	±
2000	82	100	2+	2699	465	338	2+
2053	111	111	2+	2700 †	293	338	2+
2055	82	111	2+	2701 †	360	338	2+
3275 *	152	149	1+	4008 *	400	342	3+
2186	88	160	2+	4009 *	301	342	2+
2187	122	160	2+	2639 ‡	164	316	2+
2238	130	169	3+	2640 ‡	240	317	2+
2239	115	169	3+	2702 ‡	222	338	3+

* *p*-Dimethylaminoazobenzene was added to the diet when the rats were 6 weeks old.

† These animals received 50 mgm. of nicotinic acid 3 times a week.

‡ These animals were deprived of riboflavin after 160 days.

times they were coarsely vacuolated. Some of the liver cells contained 2 or more nuclei. The cells abutting on the periportal fields as well as on the central veins were, for the most part, smaller. The Kupffer cells were prominent. Bile duct proliferation was occasionally noted. Scattered within the cellular areas mentioned above, isolated islands of liver tissue were present which did not show a striking difference from the uninvolved liver tissue, except, perhaps, in the presence of a greater number of the larger cells with more basophilic cytoplasm.

The livers graded 3+ showed carcinoma.

In the absence of riboflavin, the feeding of *p*-dimethylaminoazobenzene produced considerable hepatic damage in all animals. These changes were noted in some (not listed in the table) as early as 25 days after feeding of the dye was begun. Supplementing with nicotinic acid was ineffective in checking the liver damage caused by administration of *p*-dimethylaminoazobenzene. Likewise, the feeding of gum acacia in amounts of 0.5 cc. 3 times a week failed to influence the occurrence of pathologic changes in the liver.

The administration of 10 mgm. of riboflavin 3 times a week protected all animals from liver damage for a period of at least 4 months. However, supplementary riboflavin continuously administered failed to prevent the liver damage when the animals were exposed to *p*-dimethylaminoazobenzene for more than 5 to 6 months. Thereafter, histologic examination revealed severe involvement of the liver in all animals.

The protective effect of riboflavin in our rats receiving ample amounts of casein and choline in the basal diet consisted, therefore, in retarding the occurrence of liver damage resulting from *p*-dimethylaminoazobenzene for a period of about 5 months. During this period all animals receiving no riboflavin developed liver damage varying in degree from degenerative changes to carcinoma. After the 5 months' period of apparently complete protection, pathologic changes of the liver took place which were comparable to those observed in the riboflavin-deficient animals.

To judge from the degrees of hepatic damage noted, the initial change is of a degenerative nature which subsequently takes on a proliferative tendency terminating in carcinoma. In the prevention of liver damage, riboflavin may play its protective role by impeding the degenerative changes and thus checking indirectly the proliferative changes. It may have a dual action, since in addition to impeding degeneration, it may inhibit proliferation. This latter possibility is unlikely, however, since riboflavin neither prevents the production of benzpyrene tumors in rats (unpublished experiments of the authors) nor stops the growth of transplants of sarcoma 180 in mice (unpublished observations, Wm. Antopol and S. Glaubach).

CONCLUSION

The administration of large amounts of riboflavin (10 mgm., 3 times per week) retards the occurrence of pathologic changes in the liver produced by *p*-dimethylaminoazobenzene in rats maintained on a synthetic diet free from riboflavin and nicotinic acid; nicotinic acid does not have this effect.

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The Effect of Vitamin B_1 Deprivation on the Appearance, Growth Rate, and Course of the Jensen Rat Sarcoma

Joseph L. Jones

(From the Department of Biochemistry, University of Missouri, Columbia, Mo.)

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The relation of the vitamin B complex to tumor growth has been investigated in various types of neoplasm. Bischoff and Long (2), in studying the reaction of sarcoma 180 to terminal B avitaminosis, found the growth of subcutaneous transplants to be completely arrested or definitely retarded with attending lowered body temperature. The same investigators (1) described a retardation of growth caused by caloric restrictions *per se,* without a deficiency of specific nutrients, and later reported that vitamin B is not directly concerned with neoplastic proliferation. Brunschwig (3) found that pure brewer's yeast diets, when eaten in sufficient quantities to permit maintenance or only slight loss in weight, appear to protect against the formation of papillomatous gastric lesions, although the changes occurring in the ulcerated papillomas of the rat's stomach did not seem to be a direct result of deprivation of vitamins A, B_1, B_2, C, or D. Kensler and his associates (4), working with liver cancers produced by *p*-dimethylaminoazobenzene, found that riboflavin with casein afforded a partial protection against this tumor. However, Nakahara (8, 9) has reported that "nutritionally adequate" amounts of thiamin, pyridoxine, nicotinic acid, riboflavin, or a vitamin L concentrate either alone or combined with large additions of fish protein failed to alter the tumor incidence on a basal diet of rice and carrots. This is in agreement with the work done by Miller and his group (7) on hepatic tumors formed by *p*-dimethylaminoazobenzene, except that these investigators confirmed the protective action of diets high in riboflavin and protein. They pointed out that the protection against *p*-dimethylaminoazobenzene may not be specific for riboflavin and proteins, but may also result when high levels of other members of the vitamin B complex are fed in diets containing merely the ordinary requirements of all other dietary essentials.

The action of yeast extract on transplanted and spontaneous malignant tumors in mice was studied by Lewisohn and his coworkers (5), who reported that the injection of certain extracts of spleen and of yeast has been followed by the regression of transplanted and spontaneous mammary adenocarcinomas in several strains of mice. Pyridoxine, pantothenic acid, and biotin, when injected singly, did not influence the growth of spontaneous adenocarcinomas. In a later report on the action of various fractions of yeast extract Lewisohn (6) stated that the known vitamins of the B group did not appear to be responsible for the various activities typical of the extract.

The following paragraphs describe the effects of 3 increasing stages of B_1 deprivation on tumor growth, and the relationship of decreased body weight (brought about by caloric restriction) to tumor growth.

Three series of rats were transplanted with the Jensen rat sarcoma, obtained through the courtesy of Dr. J. W. Thompson of the United States Public Health Service. A suspension was inoculated aseptically into the gastrocnemius muscle of each rat. Each series was placed on a vitamin B_1-free diet, consisting of sucrose 57 per cent, B_1-free casein 17 per cent, B_1-free yeast 15 per cent, butterfat 5 per cent, salt mixture 4 per cent, cod liver oil 2 per cent. The normal diet was the same, except that the yeast and casein had not been rendered B_1-free. In series 1 the deprivation of B_1 was started 12 days after transplantation, *i.e.,* at a time which corresponded closely to the date of the first appearance of a tumor; in series 2 the deprivation was started on the day of transplantation; in series 3, 12 days before transplantation. For controls, animals bearing the sarcoma were kept on a normal diet and uninoculated rats, representing normal controls, were placed on the B_1-free diet and normal diet. The first appearance of a tumor was noted and its mean diameter and the weight of the host were determined every 3 days, usually until death terminated the experiment.

The results here reported represent the repetition of an experiment on approximately the same number of animals carried out during a different season, as a check on seasonal variation. Since the outcome for both seasons was similar within the limits of statistical error, individual results are reported for the more recent group and averages given for the other group, which will be referred to as the season I group.

Table I and Fig. 1 make it clear that in series 1 the growth rates for tumors in rats started on a B_1-free diet 12 days after implantation are not appreciably different from the growth rates in those on a normal diet. The time when the tumors appeared and their course are also the same for both groups. This might have been expected since the tumors on the B_1-free diet had attained almost their maximum size before the total body weight decreased or, in other words, the tumor had terminated the life of the animal before the loss of weight due to B_1 deprivation had started.

The results of starting vitamin B_1 deprivation on the same day as the transplantation (Table II and Fig. 2) are much more pronounced. The first observation to be made was that tumors appeared later on

the B_1-free diet. In the rats on the B_1-free diet 8 (rats No. 14 to 21, inclusive) reached their first recording on the 13th day and 3 others (rats No. 12, 13, and 22) by the 16th, whereas all 11 tumors in the rats on a normal diet appeared by the 10th day. It can be seen also (Fig. 2) that the tumors in the rats deprived of B_1 grew more slowly and never became as large. This slower growth rate was reflected in the longer life of the host. The survival period, after transplantation, of rats on the B_1-free diet ranged from 28 to 43 days, with an average of 36.7 days for the 11 rats in this group; while that for rats on a normal diet ranged from 16 to 34 days after transplantation, with an average of 25.2 days, giving to the B_1-free animals an average survival period 11.5 days longer than that of the controls.

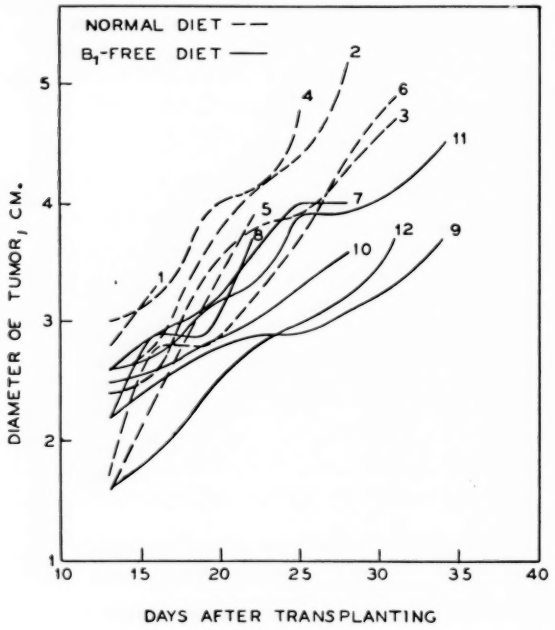

FIG. 1

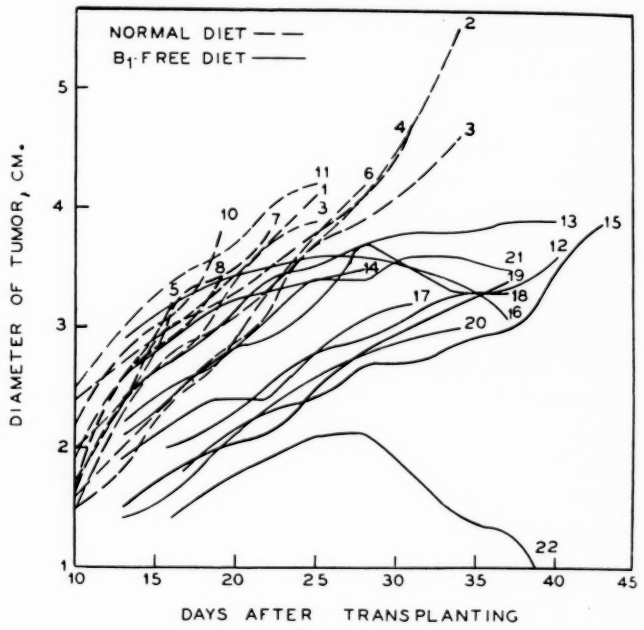

FIG. 2

FIGS. 1 AND 2.—Growth of Jensen rat sarcomas on normal and vitamin B_1-free diet.

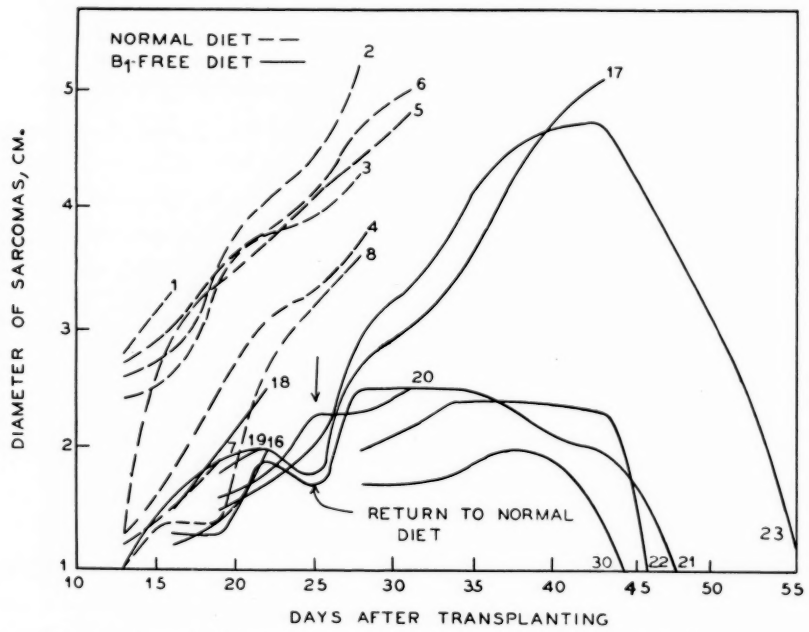

FIG. 3.—Growth of Jensen rat sarcomas on normal and vitamin B_1-free diet.

To increase the period of B_1 deprivation, series 3 (Table III and Fig. 3) was started 12 days before transplantation. Again a later appearance of the tumors was noted in the case of vitamin B_1-free rats. The 8 sarcomas in the rats on normal diet all came to observation on the 13th day. In the B_1-free group,

TABLE I: BODY WEIGHTS AND TUMOR SIZES FOR JENSEN SARCOMA RATS (SERIES 1)

Rat	Original weight	13 days	16 days	19 days	22 days	25 days	28 days	31 days	34 days
RATS ON NORMAL DIET									
BODY WEIGHT IN GM.									
1	100	136 *	156	...	...	...	...	...	...
2	144	159 *	166	175	190	201	214	...	...
3	162	186 *	198	207	216	221	235	244	...
4	172	223 *	236	247	250	256	...	...	...
5	146	190 *	205	212	215	...	...	...	...
6	124	149 *	160	164	168	173	180	186	...
Average gain by periods		33	13	8	7	7	10	8	...
Average gain season I (7 rats)		29	15	9	8	9	9	...	...
TUMOR SIZES (AVERAGE DIAMETER IN CM.)									
1	—	2.8	3.3	...	...	...	...	...	...
2	—	2.6	2.8	3.6	4.1	4.4	5.2	...	...
3	—	2.4	2.6	3.4	3.8	3.9	4.3	4.7	...
4	—	3.0	3.2	3.9	4.1	4.8	...	...	...
5	—	1.6	2.4	3.2	3.9	...	...	...	...
6	—	1.7	2.8	2.8	3.2	3.9	4.4	4.9	...
Average tumor diameter		2.4	2.9	3.4	3.8	4.3	4.6	4.8	...
Average tumor diameter season I		2.2	2.7	3.4	3.7	4.0	4.4	4.6	...
RATS ON VITAMIN B_1-FREE DIET									
(Deprivation of vitamin B_1 started 12 days after transplantation)									
BODY WEIGHT IN GM.									
7	104	142 *	157	171	174	181	181	...	...
8	112	154 *	167	171	175	...	...	...	...
9	143	197 *	208	217	228	230	230	223	218
10	167	200 *	206	219	227	232	230	...	...
11	120	144 *	152	160	164	166	159	162	161
12	167	190 *	211	229	229	227	221	213	...
Average gain by periods		36	13	11	6	3	−3	−4	−3
Average gain season I (7 rats)		31	14	9	5	4	−2	−5	−3
TUMOR SIZES (AVERAGE DIAMETER IN CM.)									
7	—	2.6	2.9	3.1	3.6	4.0	4.0	...	...
8	—	2.2	2.8	2.9	3.7	...	...	...	...
9	—	2.2	2.5	2.7	2.9	2.9	3.1	3.3	3.7
10	—	2.5	2.6	2.8	3.0	3.3	3.6	...	...
11	—	2.6	2.7	3.1	3.3	3.9	3.9	4.1	4.5
12	—	1.6	1.9	2.4	2.8	3.0	3.2	3.7	...
Average tumor diameter		2.3	2.6	3.2	3.6	3.4	4.0	3.7	4.1
Average tumor diameter season I (7 rats)		2.1	2.4	2.8	3.5	3.7	4.1	All animals dead after 28th day	

* First appearance of tumor.
— No visible tumor.
Original weight in all cases indicates weights on the day of transplantation.

TABLE II: BODY WEIGHTS AND TUMOR SIZES FOR JENSEN SARCOMA RATS (SERIES 2)

Rat	Original weight	4 days	7 days	10 days	13 days	16 days	19 days	22 days	25 days	28 days	31 days	34 days	37 days	40 days	43 days
RATS ON NORMAL DIET															
BODY WEIGHT IN GM.															
1	135	136	144	145 *	156	162	168	170	175	...	...	...	...	...	...
2	144	148	154	163 *	173	179	182	184	189	191	195	199	...	...	...
3	142	142	158	160 *	170	173	174	179	183	...	...	...	...	...	...
4	163	173	195	197 *	202	207	213	216	224	231	236	...	...	...	...
5	143	147	158	160 *	166	172	...	...	...	...	...	...	...	...	...
6	131	133	142	152 *	157	160	164	171	180	184	...	...	...	...	...
7	149	152	155	158 *	164	168	175	176	...	...	...	...	...	...	...
8	140	155	174	183 *	200	205	...	...	...	...	...	...	...	...	...
9	149	155	167	176 *	188	189	194	196	208	211	215	219	...	...	...
10	155	168	195	198 *	203	208	213	...	...	...	...	...	...	...	...
11	128	132	143	147 *	150	156	159	175	180	...	...	...	...	...	...
Average gain by periods		6	13	5	8	5	4	5	7	4	4	4	...	...	...
Average gain season I (14 rats)		15	10	7	7	6	5	3	4	4	3	3	...	...	...
TUMOR SIZES (AVERAGE DIAMETER IN CM.)															
1	—	—	—	1.7	2.6	3.0	3.3	3.7	4.1	...	...	...	...	...	...
2	—	—	—	1.5	2.3	2.8	3.0	3.3	3.7	4.1	4.7	5.5	...	...	...
3	—	—	—	1.6	2.4	2.7	3.2	3.7	3.9	...	...	...	...	...	...
4	—	—	—	2.4	2.7	3.0	3.2	3.5	3.8	4.1	4.7	...	...	...	...
5	—	—	—	1.7	2.4	3.2	...	...	...	...	...	...	...	...	...
6	—	—	—	1.5	1.8	2.4	2.7	3.3	3.8	4.2	...	...	...	...	...
7	—	—	—	2.0	2.4	3.2	3.4	3.8	...	...	...	...	...	...	...
8	—	—	—	1.9	2.6	2.9	3.4	...	...	...	...	...	...	...	...
9	—	—	—	1.6	2.0	2.4	2.8	3.1	3.7	3.9	4.2	4.6	...	...	...
10	—	—	—	2.2	2.8	3.1	3.8	...	...	...	...	...	...	...	...
11	—	—	—	2.5	3.0	3.4	3.6	4.0	4.2	...	...	...	...	...	...
Average tumor diameter				1.9	2.5	2.9	3.2	3.6	3.9	4.1	4.5	5.1	...	...	...
Average tumor diameter season I (14 rats)				2.1	2.4	2.8	3.1	3.6	3.8	4.0	4.4	4.9	...	...	...
RATS ON VITAMIN B_1-FREE DIET (Deprivation of vitamin B_1 started on day of transplantation)															
BODY WEIGHT IN GM.															
12	122	123	137	142	151	154 *	160	164	166	166	162	154	150	143	...
13	135	138	146	148	149	152 *	156	162	163	161	160	152	150	149	...
14	135	135	141	150	159 *	160	164	158	156	150	...	...	...	...	...
15	134	138	145	147	150 *	154	160	162	161	158	153	149	142	133	130
16	153	157	164	173	175 *	178	181	174	170	167	161	162	154	...	...
17	136	140	149	154	157 *	162	169	176	172	166	163	...	...	...	...
18	136	138	142	151	155 *	157	160	162	160	154	150	146	144	...	...
19	115	121	130	134	139 *	142	146	146	149	144	142	137	128	...	...
20	125	128	130	132	134 *	138	142	147	144	140	130	118	...	...	...
21	127	130	140	147	153 *	155	159	163	162	157	152	149	146	...	...
22	120	126	135	142	158	160 *	168	175	177	171	166	160	156	147	...
Average gain by periods		3	8	6	5	3	5	2	−1	−4	−5	−5	−5	−7	−3
Average gain season I (9 rats)		4	7	5	6	3	3	2	0	−3	−3	−6	−5	−3	...
TUMOR SIZES (AVERAGE DIAMETER IN CM.)															
12	—	—	—	—	—	2.0	2.2	2.5	2.8	2.9	3.1	3.3	3.3	3.6	...
13	—	—	—	—	—	2.7	3.0	3.4	3.5	3.7	3.8	3.8	3.9	3.9	...
14	—	—	—	—	2.6	2.9	3.2	3.3	3.4	3.5	...	...	...	...	...
15	—	—	—	—	1.4	1.7	2.1	2.3	2.4	2.7	2.7	2.9	3.0	3.5	3.9
16	—	—	—	—	2.9	3.2	3.4	3.5	3.6	3.6	3.5	3.4	3.1	...	...
17	—	—	—	—	1.9	2.2	2.4	2.4	2.8	3.1	3.2	...	...	...	...
18	—	—	—	—	2.1	2.4	2.8	2.9	3.2	3.7	3.5	3.3	3.3	...	...
19	—	—	—	—	1.5	1.8	2.0	2.1	2.5	2.8	3.0	3.2	3.4	...	...
20	—	—	—	—	1.4	1.6	2.0	2.3	2.6	2.8	2.9	3.0	...	...	...
21	—	—	—	—	2.2	2.5	2.7	3.1	3.4	3.4	3.6	3.6	3.5	...	...
22	—	—	—	—	—	1.4	1.7	1.9	2.1	2.1	1.8	1.4	1.3	0.0	...
Average tumor diameter					2.0	2.2	2.5	2.7	2.9	3.1	3.1	3.1	3.1	2.8	3.9
Average tumor diameter season I (9 rats)					1.8	2.1	2.3	2.6	2.8	3.0	2.9	3.0	2.9	2.9	...

* First appearance of tumor.

— No visible tumor.

TABLE III: BODY WEIGHTS AND TUMOR SIZES FOR JENSEN SARCOMA RATS (SERIES 3)

Rat	Original weight	4 days	7 days	10 days	13 days	16 days	19 days	22 days	25 days	28 days	31 days
RATS ON NORMAL DIET											
BODY WEIGHT IN GM.											
1	100	115	118	128	136 *	156	...	...	...	...	...
2	144	148	151	156	159 *	166	175	190	201	214	...
3	162	169	174	180	186 *	198	207	216	221	235	...
4	123	137	149	157	166 *	175	178	188	194	195	...
5	144	152	160	169	172 *	180	189	199	204	212	226
6	124	130	136	136	142 *	146	153	163	168	171	185
7	117	126	130	140	146 *	150	154	....	...	...	...
8	110	112	118	121	125 *	131	135	138	142	144	...
Average gain by periods		8	6	6	6	9	7	10	6	7	14
Average gain season I (6 rats)		9	5	4	5	8	6	8	7	4	...
TUMOR SIZES (AVERAGE DIAMETER IN CM.)											
1	—	—	—	—	2.8	3.3	...	...	...	...	...
2	—	—	—	—	2.6	2.8	3.6	4.1	4.4	5.2	...
3	—	—	—	—	2.4	2.6	3.4	3.8	3.9	4.3	...
4	—	—	—	—	1.3	1.8	2.5	3.1	3.3	3.7	...
5	—	—	—	—	1.2	2.9	3.4	3.7	4.1	4.4	4.8
6	—	—	—	—	2.7	3.0	3.5	3.8	4.1	4.7	5.0
7	—	—	—	—	1.2	1.5	1.9	...	...	...	...
8	—	—	—	—	1.0	1.4	2.0	2.7	3.2	3.6	...
Average tumor diameter					1.9	2.4	2.9	3.5	3.8	4.3	4.9
Average tumor diameter season I (6 rats)					1.6	1.9	2.4	2.9	3.6	3.9	...
RATS ON VITAMIN B_1-FREE DIET (Deprivation of vitamin B_1 started 12 days before transplantation)											
BODY WEIGHT IN GM.											
16	113	107	99	99	97	90 *	86 *	84	Died	...	...
17	130	121	114	108	105	104	100	99	90	...	...
18	127	119	110	105	100	94 *	92	95	Died	...	...
19	139	127	118	112	105 *	103	99	92	Died	...	...
20	147	142	140	138	130	127	120 *	113	114	...	...
21	164	155	150	149	149	144 *	139	131	123	...	...
22	167	158	153	152	144	134	135	131	126	...	...
23	196	172	176	169	161	150	145 *	148	133	...	...
30	96	101	102	101	99	95	90	93	94	...	...
Average loss by periods		— 9	—4	—3	—5	—5	—4	—2	—6	...	...
Average loss season I (8 rats)		—12	—7	—4	—5	—4	—4	—3	—7	...	...
TUMOR SIZES (AVERAGE DIAMETER IN CM.)											
16	—	—	—	—	—	1.2	1.4	2.0	Died	...	...
17	—	—	—	—	—	—	1.5	1.7	2.0	...	...
18	—	—	—	—	—	1.5	2.0	2.5	Died	...	...
19	—	—	—	—	1.0	1.6	1.9	2.0	Died	...	...
20	—	—	—	—	—	—	1.6	1.8	2.3	...	...
21	—	—	—	—	—	1.3	1.3	1.9	1.7	...	...
22		Tumor did not appear until after return to normal diet									
23	—	—	—	—	—	—	1.8	2.0	1.8	...	...
30		Tumor did not appear until after return to normal diet									
Average tumor diameter					1.0	1.4	1.6	2.0	1.9	All animals dead	
Average tumor diameter season I (8 rats)					—	1.1	1.3	1.5	1.6	before 28th day	

* First appearance of tumor.
— No visible tumor.

TABLE III—*Continued*

REMAINING RATS AFTER RETURN TO NORMAL DIET †

Rat	28 days	31 days	34 days	37 days	40 days	43 days	46 days	49 days	52 days	55 days
					BODY WEIGHT IN GM.					
17	123	139	159	186	205	207	...	...	...	..
20	113	196	...	...	...	...	...	...	...	...
21	163	170	191	198	204	213	217	220	...	...
22	175	190	208	222	224	228	228	231	...	...
23	165	190	214	241	256	263	255	252	250	255
30	123	132	146	160	167	171	160	157	...	...
Average gain by periods.	30	26	19	18	10	5	−4	0	−2	5
					TUMOR SIZES (AVERAGE DIAMETER IN CM.)					
17	2.7	3.0	3.4	4.2	4.8	5.1	...	...	...	...
20	2.3	2.5	...	...	...	...	...	...	...	...
21	2.5	2.5	2.5	2.4	2.1	2.0	1.5	0.0	...	...
22	2.0	2.2	2.4	2.4	2.4	2.3	0.0	...	2.4	1.2
23	2.9	3.0	3.9	4.5	4.8	4.7	4.0	3.3	...	...
30	1.7	1.7	1.8	2.0	1.9	1.4	0.0	...	...	...
Average tumor diameter.	2.4	2.5	2.8	3.1	3.2	3.1	1.4	1.7	2.4	1.2

† Season I group was not returned to normal diet since all animals had died.

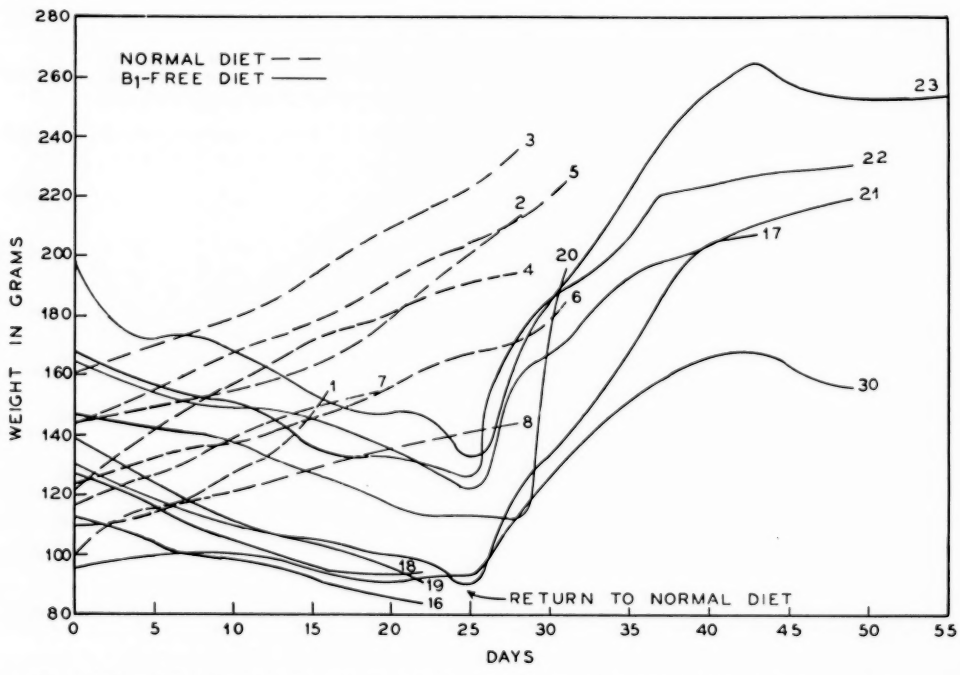

FIG. 4.—Body weights of Jensen sarcoma rats on normal and B_1-free diets and return of B_1-free rats to normal diets.

however, only 1 tumor (rat No. 19) appeared on the 13th day. Three others (rats No. 16, 18, and 21) appeared by the 16th day, and 3 more (rats No. 17, 20, and 23) on the 19th day. In this group it is interesting to note that 2 tumors did not appear until 3 days after the animals had been restored to a normal diet.

A longer duration of life after the first appearance of the tumor is noticed in this B_1-free group. Of the 9 rats, 3 (Nos. 16, 18, and 19) died in 23 days, primarily from extreme B_1 deprivation. The remaining 6 were returned to normal diet 25 days after transplantation.

This resumption of normal diet was interesting in respect to its effect upon the future course of the tumor. To begin with, a sharp increase in both body weight and tumor growth rate immediately followed and, as previously stated, 2 sarcomas (rats No. 22 and 30) appeared on the 28th day after transplantation. That the severe B_1 deprivation had a pronounced effect on the future course of the tumor is evidenced by the

fact that 4 of the 6 remaining sarcomas completely regressed and disappeared (rats No. 21, 22, and 30); the other 2 rats died. The tumor in rat No. 23 was an especially remarkable example, for regression set in after it had attained a size of 4.8 cm.

The factor of caloric restriction, due to B_1 deprivation, has to be considered. When Fig. 4 is compared with Fig. 3 it is evident that the weight curves of the animals on B_1-free diets decreased in a parallel fashion with the growth curves for their corresponding sarcomas. It should be noted here that the average gain in weight of the animal is being compared with a linear dimension, *i.e.* the diameter of the corresponding tumor (Fig. 3). When these rats were returned to normal diet after the 25th day following tumor implantation, the weight curves of the animals and the growth curves for the sarcomas sharply increased in a parallel fashion only for the first few days. As the rats grew and continued to increase their caloric intake 3 of the sarcomas (rats No. 21, 22, and 30) started to recede and finally disappeared. A short while later another one (rat No. 17) began to regress. In other words, in spite of the increased caloric intake and weights of the rats, these 4 sarcomata were retarded, then began to regress, and finally disappeared. It is, therefore, reasonable to assume that these effects are due to the vitamin B_1 deprivation rather than to the caloric changes.

SUMMARY AND CONCLUSIONS

The effect of vitamin B_1 deprivation on the time of appearance, growth rate, and course of the Jensen rat sarcoma was studied at 3 stages of deprivation; namely, (a) deprivation started when the tumor appeared, 12 days after transplantation, (b) deprivation started on the day of transplantation, (c) deprivation started 12 days before transplantation with return to normal diet 25 days after transplantation.

1. Tumor growth rates were not affected by deprivation started 12 days after transplantation. This was accounted for by the fact that the tumor destroyed the animals before the deprivation had had any serious effect.

2. Deprivation started on the day of transplantation slowed the tumor growth rate and thereby increased the total survival period of the host.

3. Deprivation started 12 days before transplantation resulted in a still later appearance of the tumor; it also decreased the growth rate of the tumor and decreased its size, thus increasing the survival time still further.

4. The return of the B_1-free tumor-bearing rats to a normal diet 25 days after transplantation temporarily increased the tumor growth rate together with the body weight.

5. B_1 deprivation had an effect on the subsequent course of the tumor as shown by the later appearance and by regressions after return to a normal diet.

6. B_1 deprivation, quite apart from caloric restriction, affected the future course of the tumor.

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Three Carcinomas of the Tongue in Two Monkeys

Paul E. Steiner, M.D., Heinrich Klüver, Ph.D., and Alexander Brunschwig, M.D.

(From the Department of Pathology, the Otho S. A. Sprague Memorial Institute, and the Department of Surgery, The University of Chicago, Chicago, Ill.)

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The factors predisposing to carcinoma of the tongue in man are stated to be: (a) trauma from broken teeth or poorly fitting dentures, (b) the use of tobacco, especially pipe-smoking, (c) the ingestion of excessively hot foods, (d) syphilis, (e) vitamin deficiency, and (f) heredity. The simultaneous occurrence of 3 carcinomas of the tongue in 2 monkeys which apparently had none of these factors is of interest. In its clinical course and its gross and microscopic pathology the disease resembled that seen in man. Furthermore, as is usual in man, the victims were elderly males.

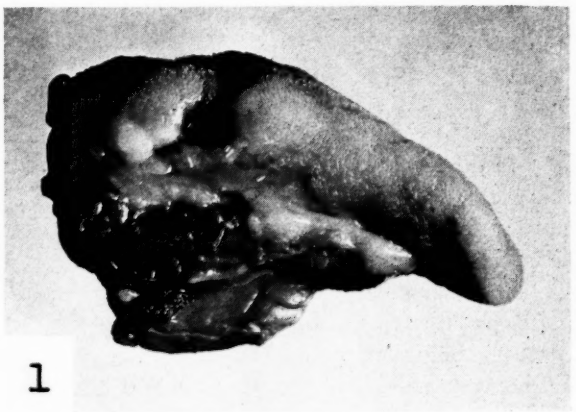
Among primates carcinoma of the tongue appears to be a disease of man exclusively. It is not mentioned in the reports on tumors in the lower primates by Fox (5) and by Ratcliffe (12) from the Philadelphia Zoo, in that of Zuckerman (14) from the London Zoological Society, or in those of Kennard (8) and Kennard and Willner (9). In the upper alimentary tract, Zuckerman (14) reported a case of carcinoma of the floor of the mouth in a rhesus monkey, and Fox (6) described a squamous cell carcinoma of the esophagus in a Japanese macaque.

Carcinoma of the tongue is not mentioned in the book on neoplasms of domesticated animals by Feldman (3) or in the review by Wells, Slye, and Holmes (13) of the comparative pathology of cancer of the alimentary canal, including more than 142,000 necropsies in the Slye mice. Cadiot (2) collected 7 cases of carcinoma of the tongue in domesticated animals from the older literature, including 3 in horses, 2 in cats, and 1 each in a cow and a dog. To these Hartig (7) added 2 cases in horses, 3 in cows, and 1 in a dog all collected from the literature, together with various types of connective tissue tumors. There appears to be no case in animals recorded in the American literature. The extreme rarity of this tumor among American meat-producing animals is shown by Bengston (1) who writes ". . . . our records show that only one such case has been encountered since 1906. This occurred in a ten year old cow in 1922. It was reported that there was a large well-organized growth at the base of the tongue and that the submaxillary, atlantal and parotid lymph glands contained tumor growths. Microscopic examination showed squamous cell carcinoma in the lesions on the tongue and in the involved lymph glands." His experience covers the large autopsy service at the stockyards in Chicago.

Case Reports

These 2 monkeys were members of a colony of Old World and New World primates kept by one of us (H. K.) for long range neurophysiological and behavior studies (10, 11).

Monkey No. 1.—A male rhesus monkey (*Macaca mulatta*), approximately 16 years old, under study in the laboratory since February, 1934, first developed difficulty in swallowing solid food on Jan. 22, 1942. Weight 15¼ pounds. Examination 2 days later revealed a deep ulcer along the right lateral border of the tongue, at the junction of the middle and posterior thirds. It had a rolled, hard, white margin and a clean floor. Biopsy on Jan. 27, 1942, disclosed a squamous cell carcinoma. Under nembutal anesthesia, on Feb. 4, 1942, the anterior three-fourths of the tongue was surgically amputated. The postoperative course at first was uneventful but eating was not resumed. On one occasion when a stomach tube was being passed the trachea was inadvertently entered and food was poured into the lungs. Death occurred on the 8th postoperative day.



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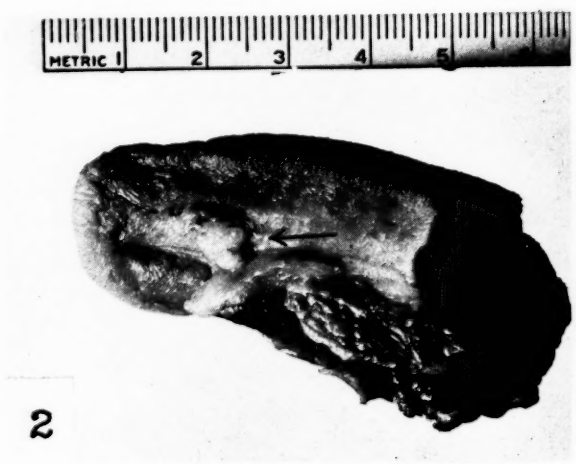

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Fig. 1.—The ulcerated tumor on the lateral margin of the tongue in monkey No. 1.

Fig. 2.—The tumor on the under surface of the tongue adjacent to the frenulum in monkey No. 1.

Operative specimen: The tongue in general was soft and pliable and had a thin epithelium. On its right lateral border, beginning 4.2 cm. from the tip and extending back, was a hard, pearly white tumor which measured about 23 × 11 mm. At its center was a deep ulcer which was 13 mm. long and from 1 to 5 mm. wide. Its margins were rolled and hard and about 5 mm. wide (Fig. 1). On the cut surface the pearly gray-white tumor was seen to extend into the tongue to a depth of 4 mm. beneath the floor of the ulcer and up to 7 mm. at its margins.

On the ventral surface of the amputated tongue to the left of the frenulum was a second tumor. It was a white, firm, slightly mammillated papilloma which measured 9 × 7 mm. in diameter and was elevated to a maximum of 2 mm. (Fig. 2). On cutting through this tumor no appreciable gross extension into the muscle of the tongue was seen.

Microscopic examination of the larger tumor showed an infiltrating squamous cell carcinoma. It was not highly undifferentiated although there was little keratinization. Groups of tumor cells penetrated into the muscle up to 6 mm. beneath the surface at the levels studied (Fig. 5). The smaller, papillary tumor was also a squamous cell carcinoma. It, too, was a fairly well differentiated tumor but with only slight keratinization. Its cells penetrated deeply into the muscle. They were seen up to 6.5 mm. beneath the surface (Figs. 3 and 4).

Autopsy: The postmortem examination disclosed no residual cancer in the tongue and no metastases. The principal abnormality was much food material in the bronchial system with some pulmonary edema and focal congestion. Emaciation was moderate; the weight was now 11½ pounds.

Monkey No. 2.—A male Java monkey (*Macaca irus*), about 14 years old, under observation in the laboratory since July, 1933, developed lassitude and difficulty in swallowing solid food in November, 1941. Examination disclosed an ulcer on the left margin of the tongue. This did not heal when gentian violet and other local medication was applied but enlarged progressively so that eventually a deep, transverse ulcer, which bled easily, extended across the tongue and over the adjacent pharyngeal surfaces. The animal, which had been unusually healthy in appearance, lost weight rapidly, although some liquids were taken. A biopsy made from the margin of the tongue ulcer on Jan. 27, 1942, disclosed squamous cell carcinoma. The monkey died on Feb. 4, 1942.

Autopsy: Autopsy disclosed definite emaciation (weight 6½ pounds), extensive bilateral aspiration pneumonia with multiple lung abscesses, and left empyema. The tongue was shortened, fixed in position, and partly amputated by a large, deep ulcer which covered its posterior third (Fig. 6). This extended posteriorly to the epiglottis, and laterally over the pharyngeal walls and the adjacent hard and soft palates. The floor and margins of this ulcer were hard and showed grayish-white tumor tissue. The floor of the mouth was rigid and the tongue was firmly adherent by tumor tissue to the right ramus of the mandible over an area about 2 cm. in length.

Microscopic examination showed that the tumor was an undifferentiated, widely infiltrating, squamous cell carcinoma with some areas of keratinization (Fig. 7). It involved much of the tongue and infiltrated the floor of the mouth, the upper end of the esophagus, the larynx, the lateral and posterior pharyngeal walls, the upper trachea, the capsule of the thyroid gland, and the submaxillary salivary glands. One submaxillary lymph node showed metastatic carcinoma. There were no metastases below the neck. One section taken through the lower lip showed an area of leukoplakia (Fig. 8). This had not been noticed upon gross examination.

Etiological Studies

The almost simultaneous appearance of 3 cancers of the tongue in 2 monkeys led to a search for possible etiological factors. Although these studies yielded no positive information they are placed on record.

Hereditary factor.—A common hereditary factor seems very remote for the following reasons: The monkeys were of different species of the genus *Macaca.* They were purchased from different dealers in New York City at different times—one in July, 1933, and the other in February, 1934. They probably originated in different countries. Thus while members of these species can interbreed successfully it is highly improbable that these 2 monkeys were born of interspecific crosses.

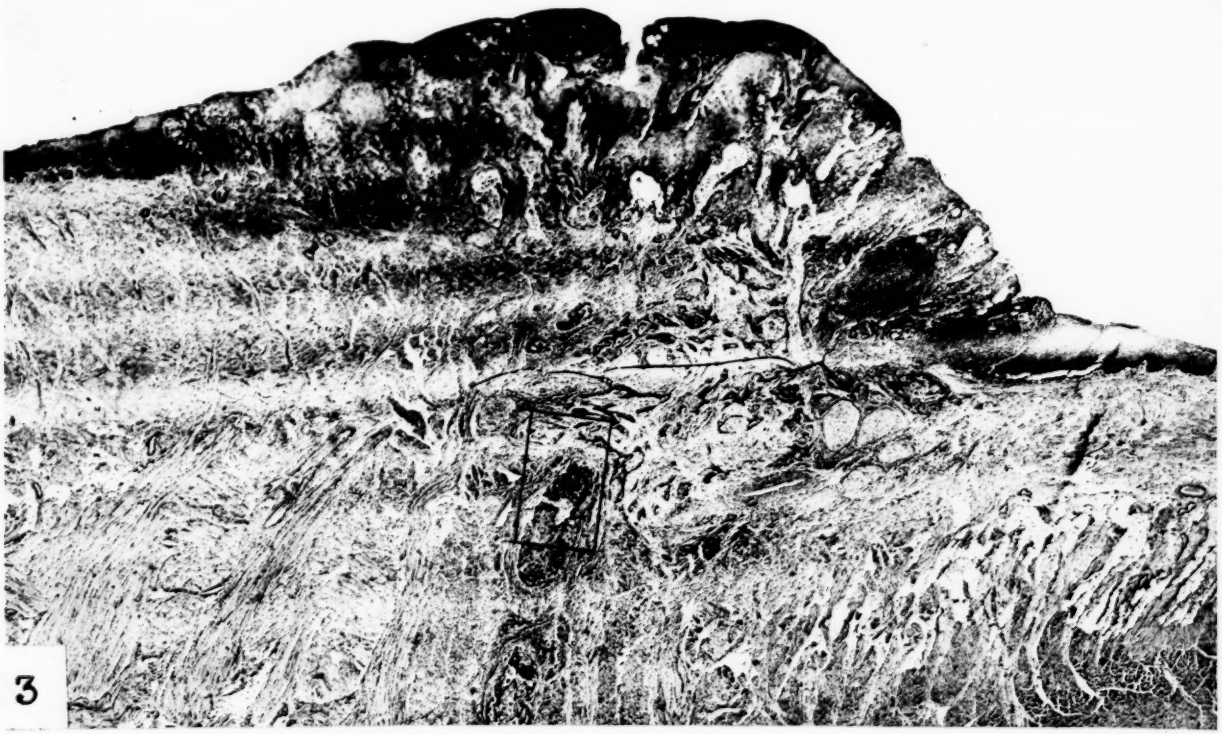
Brain lesions.—One factor which these monkeys had in common was experimental bilateral cerebral lesions, although they were in different locations. Monkey No. 1 had a left occipital lobectomy in June, 1937, and a right occipital lobectomy in September, 1937. Monkey No. 2 had bilateral removal of the prefrontal lobes in August, 1935. The removals were made with a knife under pentobarbital sodium anesthesia supplemented by ether. Other members of the colony having had similar operations at about the same time are in good health.

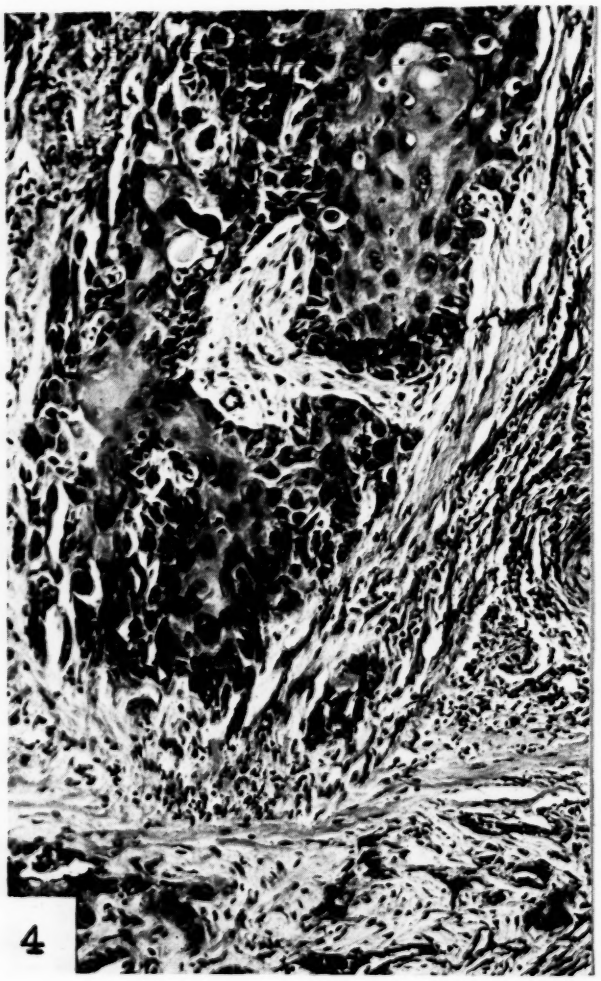
Hormonal and sex factors.—Both monkeys were males. Monkey No. 1 was the father of a female baby born in April, 1935, and still living. He showed repeatedly a pronounced reddening of the scrotum and skin of the face, especially between the eyes, in 1939. This occurred again in 1940 and 1941. A similar reddening has not been observed in other male rhesus monkeys and other macaques in this colony. Gross and microscopic examinations of the genital tracts of both monkeys at autopsy showed that they were not sexually senile.

Age factor.—The approximate ages at death were 16 and 14 years respectively. In view of what is known about the duration of life of members of the genus *Macaca,* both must be considered aged (4).

Diet factor.—The diet fed this colony has apparently been adequate as judged by longevity, freedom from disease, general condition, learning ability, and reproduction. Births have frequently occurred and even South American monkeys have thrived. For example, 3 births have occurred during the past 2 years in night monkeys (*Aotes*), to our knowledge the only such births in captivity.

The monkeys are fed one main meal a day consisting of fruits and vegetables, white bread, milk, and water. This is supplemented by peanuts, sunflower seeds, cod liver oil, sugar, wheat germ, and chimcrackers at other times of the day. Depending on seasonal availability the fruits and vegetables fed were: bananas, apples, pears, oranges, tangerines, grapefruit, grapes, watermelons, honeydew melons, carrots, pineapples, cantaloupes, peaches, tomatoes, potatoes, lettuce, spinach, cabbage, squash, sweet potatoes, green beans, yellow corn, green peas, peppers, plums, pumpkins, cucumbers, onions, rutabagas, lemons, cauliflower, asparagus, celery, rhubarb, and eggplant.





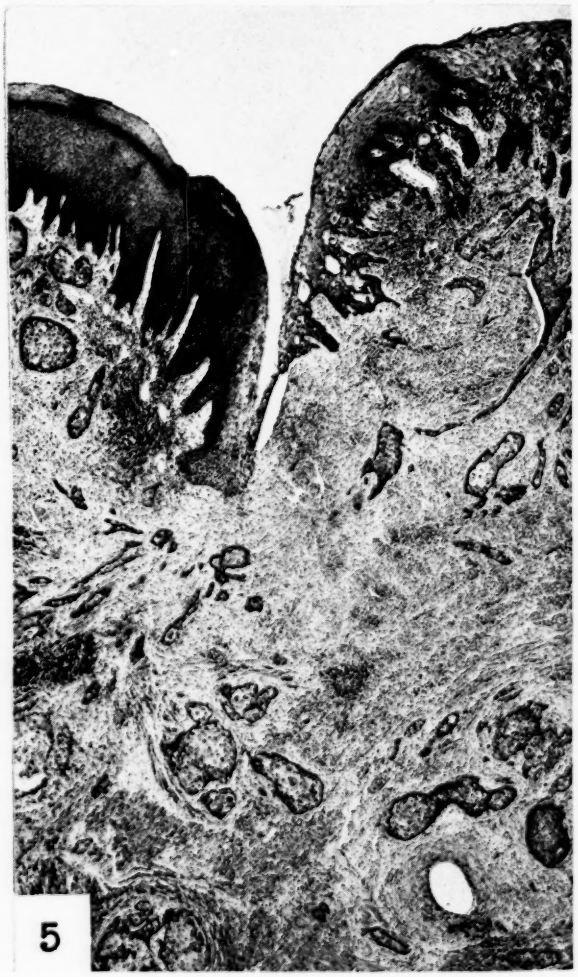
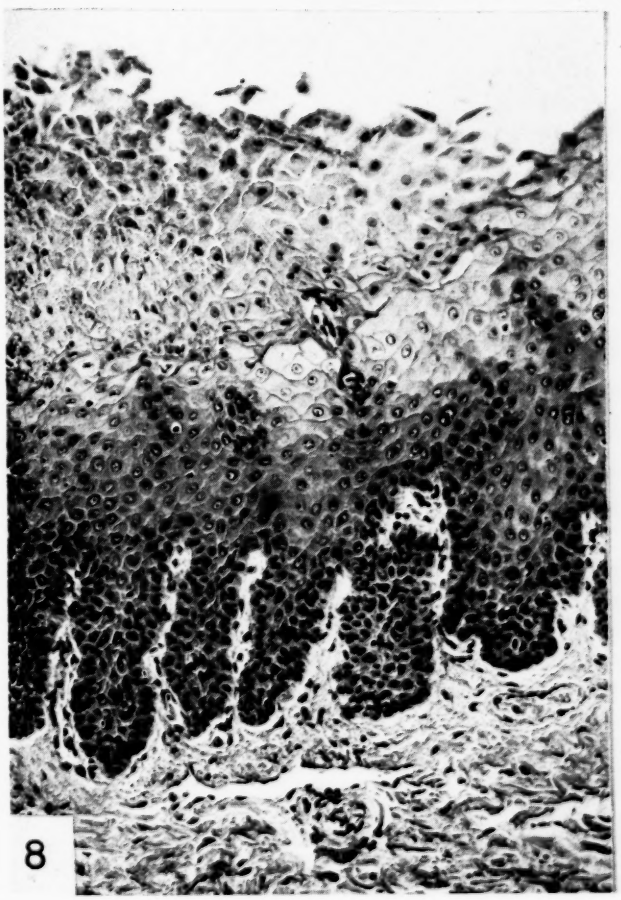


FIG. 3.—The tumor of Fig. 2, showing penetration of groups of abnormal epithelial cells deep into the muscle. Mag.×17.

FIG. 4.—Higher power of the field marked in Fig. 3. Mag.×120.

FIG. 5.—The tumor shown in Fig. 1. Mag.×23.



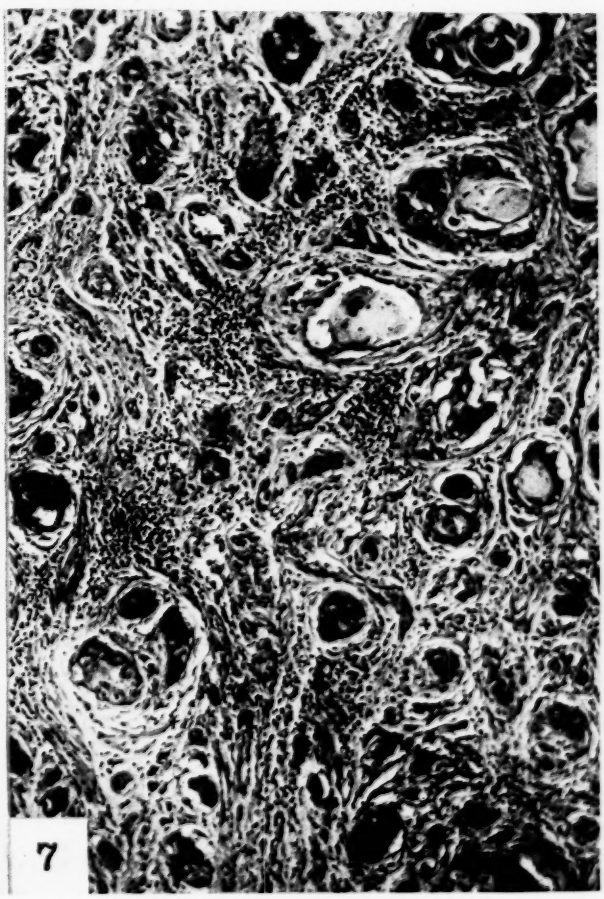


FIG. 6.—A sagittal section through the tongue and floor of the mouth from the tip to the larynx in monkey No. 2. Ulceration of the tumor with deep penetration is shown. Mag.×4½.

FIG. 7.—The structure of the tumor in Fig. 6. Mag.×100.

FIG. 8.—The margin of an area of leukoplakia in the mouth of monkey No. 2. Mag.×150.

Chimcracker formula:

40.8 per cent 2nd clear wheat flour
16.5 per cent soybean meal
8.7 per cent corn meal
4.85 per cent ground wheat
4.85 per cent milk powder
4.85 per cent ground raisins
4.85 per cent bone meal
4.85 per cent salad oil
3.9 per cent molasses (blackstrap)
0.6 per cent salt
4.3 per cent wheat germ
1.0 per cent irradiated yeast

Except for the leukoplakia of the lip in monkey No. 2, discovered incidentally and illustrated in Fig. 8, no evidence for any vitamin deficiency was found. In this animal the poor nutrition for 3 months consequent to the inability to swallow may well account for this change.

Infectious and contagious factors.—These 2 monkeys were housed on different floors of the same building, during the past 6 years, with other monkeys which have remained well. They had, however, the same caretakers and the same experimental staff who used no aseptic precautions. There have been occasional colds and a few single cases of amebic dysentery. Tuberculosis was last seen 8 years ago and that among newly purchased animals. Monkey No. 1 was the cleanest and most fastidious animal seen in the colony in the last 10 years.

Filtration experiment.—The 2 portions of the papillary carcinoma (illustrated in Figs. 2, 3, and 4) which remained after a block of tissue was taken entirely through its center for the microscopical examination, were dissected from the tongue. This tumor tissue weighed about 200 mgm. It was minced with scissors in 5 cc. of physiological salt solution, after which it was ground in a mortar. Half of the saline was then passed through a Seitz filter. Small portions of this filtrate were injected into the mucous membrane at the lateral margin of the tongue of another monkey (*Macaca mulatta*). Examination of the tongue of the latter 3½ months later revealed no abnormality.

Other environmental factors.—These animals were used for behavior studies before and after the brain operations. Monkey No. 1, for example, was tested daily except on week ends from April, 1936, to December, 1941. Thus they were under constant close observation.

The temperature in the laboratory is kept at 75-80° F. during the winter. The rooms receive daylight but sunlight hardly ever reaches the animals and that through windowpanes. Artificial radiation is never employed.

The cages are of metal and chemically untreated wood. Wooden shavings, also untreated by chemicals according to the dealer, are used on the floor. The food pans are aluminum or enamel. Other metals with which the animals have had contact are iron, brass, tinned copper braid, and galvanized iron. The cages and food pans are cleaned with hot water, no chemicals being used. No drugs other than the anesthetics mentioned were ever given. No injuries of the tongue were ever noted. Other animals, at times, in the laboratory were: cat, mice, rats in cages, cockroaches, men.

These monkeys showed no gross or microscopical evidence of vascular or central nervous system syphilis. They were not addicted to the use of tobacco. They were never given hot or boiled food or drink. Dental plates or other dentures were not worn. Their teeth were in good alignment and there were no palpable rough spots or visible caries.

SUMMARY

Three squamous cell carcinomas of the tongue occurred simultaneously in 2 macaque monkeys (*Macaca mulatta* and *Macaca irus*) in a primate colony. One monkey died of aspiration pneumonia with local tumor metastases, and the other died postoperatively without metastases. Attempts at transmission were made and remain negative after 3½ months. None of the commonly quoted etiological factors for cancer of the tongue in man were present except that both were elderly males. Both had experimental brain lesions of long standing. This is the first report of cancer of the tongue in subhuman primates, and also the first in nondomesticated animals.

ADDENDUM

The case of carcinoma of the tongue in a horse reported by Henson (J. Am. Vet. M. A., **94**:124. 1939) has come to our attention since the above was written. Also, we have seen microscopic sections from a third instance of carcinoma of the tongue in a macaque. This was discovered in a newly purchased animal in 1940 by Dr. Elizabeth Hemmens of the Department of Bacteriology, The University of Chicago.

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Possible Relationship of the Estrogenic Hormones, Genetic Susceptibility, and Milk Influence in the Production of Mammary Cancer in Mice*

John J. Bittner, Ph.D.

(From the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

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At least three etiological factors have been recognized in the development of spontaneous mammary tumors in mice. These are: the inherited susceptibility or nonsusceptibility of the individual, sufficient hormonal stimulation of the mammary gland, and some influence in the milk which the animals receive while nursing. Others may be involved but have not been identified.

I. THE ROLE OF THE HORMONES

A. Introduction

"Carcinoma can occur only in a mammary gland which has undergone a certain degree of development. The development of the mammary gland is dependent upon the estrogenic and pituitary hormones. But whether these stimulating factors play a direct role in the cancerization process, or only produce the anatomical development of the mammary gland sufficient to allow the cancer process to manifest itself is still undetermined."—from Lacassagne (36).

Earlier it had been demonstrated by Loeb (39, 40), Cori (25), and Murray (43–46) that few tumors of the mammary gland developed in mice which had been ovariectomized. In general, the younger the mice were when spayed the lower the incidence of mammary tumors.

Woolley, Fekete, and Little (62–64) have recently observed that castration in strains of mice having a high incidence of mammary tumors may be followed by hyperplastic changes in the adrenal cortex. In the females stimulation of the uterus, vagina, and mammary glands was noticed. Mammary tumors developed in mice of both sexes and it was concluded that the hyperplastic adrenal may directly or indirectly simulate or replace ovarian activity in stimulating the growth of the mammary tissue and the development of mammary cancer.

The significance of the estrogenic [1] hormones in the development of mammary tumors became still clearer when Lacassagne (35) found that tumors might appear following the injection of these hormones over an extended period. In some experiments carcinomas arose in both males and females. Gardner (28) and Loeb (41) have recently reviewed the literature in this important and growing field.

B. The Structure of the Mammary Glands

Several workers have studied the structure of the mammary glands of mice to determine if there might be some anatomical structural relationship associated with the development of neoplasia.

Gibson (33) found that the mammary glands of mice of a strain which developed tumors were in-

* Presented at the Endocrine-Cancer Conference, Atlantic City, June 5, 1942.

[1] The use of this term implies that, unless indicated, the pituitary hormones are also active (34).

clined to show metaplasia of the epithelial elements, rather than atrophy, as age advanced. Mice of a strain producing few tumors had glands which underwent involution and fibrosis in an orderly and normal manner.

Gardner and Strong (31) could find no significant structural difference in the mammary glands of virgin females of strains varying in their predisposition to mammary cancer.

In mice of the dilute brown stock (high incidence) and the C57 black strain (low incidence), Fekete (26) observed variations in the mammary glands. Animals of the strain which developed few tumors had uniform changes in the epithelium of these glands during the latter part of pregnancy; as function was discontinued their cells ceased to secrete and the alveoli regressed completely. The cells of the glands of mice belonging to the dilute brown (high tumor) stock, on the other hand, did not always stop multiplication toward the end of pregnancy; some continued to multiply. During regression of the glands small groups of alveoli often persisted and some cells continued to secrete. This persistent mitotic activity in small groups of cells probably marked the site of early malignant changes.

Gardner, Strong, and Smith (32) likewise noticed that the mammary glands from mice of strains with different susceptibilities showed anatomical characteristics which could be associated with neoplastic changes. Localized hyperplastic nodules were more common in mice of strains having a high incidence of mammary tumors. The authors did not believe that the occurrence of these nodules indicated greater ovarian stimulation of the glands in mice from strains having many tumors but thought that the alteration might be a result of "factors passed from parent to offspring in association with other factors predisposing to cancer." The number of hyperplastic nodules tended to increase with the age of the animals.

Injecting theelin into normal male mice of different strains, Gardner, Diddle, Allen, and Strong (30) determined that even mammary rudiments could be made to respond to the stimulation. In general there was no strain variation.

Gomez, Turner, Gardner, and Hill (34) found that growth of the mammary glands of hypophysectomized mice did not occur following the injection of estrogens, whereas the glands of nonhypophysectomized male or ovariectomized immature female mice developed rapidly under treatment. This study confirmed the observations of Nelson and Pfiffner (49).

Gardner, Strong, and Smith (32) concluded that since the mammary glands of mice would not respond beyond a certain point to continued treatment with estrogenic hormones, some factor or factors maintained them in equilibrium with other tissues or organs.

van Gulik and Korteweg (59) observed that the characteristic architecture of the mammary glands of mice of the dilute brown (high cancer) and the C57 black (low cancer) stocks might be altered as a result of foster nursing. Foster nursing on females of the resistant strain decreased the proliferation of the gland-tree and lowered the incidence of tumors; on the other hand, hyperplastic nodules were observed in the glands of mice belonging to the C57 black strain and nursed on females of the dilute brown stock. These authors believe that the architecture of the mammary glands is probably determined by both intrinsic factors and the type of milk received. Their observations have been confirmed by Shimkin, Grady, and Andervont (51) on mice of the C3H and C stocks.

Fostered mice of the A stock will develop mammary tumors if nursed on females from strains with a high incidence of mammary tumors, or if artificially fed, when 4 to 6 weeks of age, with milk from females of strains with a high incidence (15). If fostered mice are permitted to have several litters before they are given the milk with the active influence, apparently few mammary tumors will result (Duran-Reynals and Bittner, unpublished). Thus, the active milk influence may have to be present from the time the mammary glands start to develop in order to prepare them for the cancerous change.

C. Variations in the Degree of Estrogenic Stimulation in Different Inbred Strains of Mice

Inbred strains of mice which differ considerably in tumor incidence are being used for studies on the etiology of mammary tumors. Some develop few tumors (37); some have a low incidence in virgin females and a high incidence in breeding females (13), whereas other stocks have a high incidence in both virgin and breeding females (4, 5, 59). The greatest variations in susceptible stocks have been observed in C3H and A mice, virgin females of the C3H strain having a very high incidence (4–6) and virgin females of the A stock a very low one (13), whereas breeding females of the two strains have a high incidence. Mammary tumors develop at an earlier average age in breeding females than in virgin females of the C3H stock, an observation suggesting that the amount of estrogenic stimulation has some bearing on the time at which the normal gland may change to a cancerous gland.

It is of interest to speculate on the cause or causes of the difference in tumor incidence for virgin females of the A and the C3H strains, and it may be suggested

that the high incidence of mammary cancer in virgin females of the latter, as compared with the low incidence for virgin mice of the A strain is perhaps the result of:

(a) An increased production of estrogenic hormones.

(b) A decrease in the rate of destruction of these hormones.

(c) A lower threshold sensitivity of the mammary tissue to neoplastic change.

It was found by van Gulik and Korteweg (58) that the degree of response of the genital organs to follicular hormones was characteristic for each of the five strains tested, and they thought it probably determined by intrinsic factors. The mice of the two strains with a high incidence of spontaneous mammary tumors showed very little response, probably because, it was concluded, they produced naturally a relatively large amount of the hormones. The authors suggested that if the mammary glands from strains of different incidence are equally susceptible to follicular hormones, the excess amount of such hormones secreted by mice of strains with a high incidence of mammary tumors might increase the probability that their glands would become cancerous. Thus there might exist a causal relation between the amount of the hormones secreted, as expressed by the low susceptibility of the genital organs to the hormones, and the disposition to mammary cancer.

Shimkin and Andervont (50), however, using control and foster nursed mice of strains with different incidences, found that regardless of changes in the incidence resulting from foster nursing the susceptibility of the different strains to estrone was not altered and thus was not causally related to the development of spontaneous mammary cancer. Variations in the response to estrone of mice from different strains are *strain* differences and, as such, are probably caused by inherited factors. Thus the amount of estrogenic hormones secreted by virgin females of different strains would be determined by heredity, and the incidence of mammary tumors would be higher in virgin females of the C3H stock than in virgin females of the A stock because inherited characteristics produced a higher level of estrogenic stimulation of the mammary tissue. This difference has also been observed in the response of mice of these strains to the artificial administration of estrogens (27, 42).

If the amount of estrogenic hormones secreted by virgin females of various strains is the result of intrinsic (genetic) factors, mutations might alter this amount, change the degree of mammary stimulation, and so result in variations in the incidence of mammary carcinoma. This suggestion is supported by experimental observations.

Thus in 1928 Murray (44) reported that the occurrence of mammary tumors in virgin females of the dilute brown stock was 15.8 per cent; by 1941 (47) it had increased to 61.6 per cent, and mice from the same strain have lately shown an incidence of 85 per cent in virgin females raised in another laboratory (58). Again, virgin females of the A stock had an incidence of 4.9 per cent in 1939 (13) and Murray (48) has recently reported that the virgin females of his A stock now have a high incidence of mammary tumors.

The production of estrogenic hormones may be influenced by diet. Bagg (8) reported that it was necessary to supply a high protein diet to keep the animals breeding actively in his forced breeding experiments, and Visscher and his associates (61, 7) have recently demonstrated that the incidence of mammary tumors may be decreased by restricting the caloric intake. Definite changes in the structure of the ovaries and mammary glands were described, probably resulting from a reduction in the amount of estrogenic hormones secreted by the experimental animals. When an attempt was made to breed some of the mice it was noted that less than 40 per cent of the females were fertile and that few of these gave birth to more than one litter (61).

The amount of estrogenic stimulation of the mammary tissue may be further increased: (a) as the result of normal breeding, permitting the females to nurse their offspring; (b) by forced breeding, the functional test of Bagg (8–10), where the young are removed as soon after birth as possible and the mothers are returned to the breeding pens, to become pregnant usually within a few days; and (c) by the administration of estrogenic hormones (35). If the amount of hormones secreted in the virgin females of different strains may possibly be the result of intrinsic factors, the increased secretion of such hormones associated with breeding and injection would be a combination of intrinsic and/or extrinsic causes.[2] The effects of an increased amount of estrogenic stimulation are apparent in causing mammary tumors to appear at an earlier average age. For example, Andervont (4) found that tumors developed in virgin females of the C3H stock at an average age of 10.4 months, whereas the normal breeding females developed tumors at an average age of 8.6 months.

The largest amount of hormones may be produced as the result of forced breeding since Gardner (29) determined that "the environment of mammary glands in females subjected to continuously repeated pregnancy predisposed to a higher incidence of mammary

[2] Pregnancy is here listed as due to an extrinsic cause; namely, the introduction of sperm.

tumors than did the environment created by administration of estrogens."

D. Summary

The amount of estrogenic hormones produced in virgin females of various stocks is the result of strain differences and as such may result from intrinsic factors. In some susceptible strains of mice the amount of hormones secreted by virgin females will produce a sufficient development of the mammary tissue to result in a high incidence of mammary tumors. Other strains of susceptible mice have a low incidence in virgin females probably because their intrinsic factors are able to produce only a low level of estrogenic stimulation. The production of estrogenic hormones may be further increased as the result of extrinsic causes—the production of young, as in the case of normal breeding; forced breeding; or the injection of hormones over a long period of time. In susceptible mice the average tumor age is correlated with the amount of estrogenic stimulation, and in nonsusceptible animals with the active milk influence the incidence of mammary tumors and the average tumor age are also related to the level of estrogenic stimulation.

The amount of estrogenic stimulation may be influenced also by diet.

II. CHARACTERISTICS OF THE ACTIVE MILK INFLUENCE

In 1933 it was determined that some hitherto unrecognized maternal adjuvant also was instrumental in the development of mammary cancer in mice (52). This was soon discovered to be an active "influence" present during the entire lactation period in the milk of females from strains with a high incidence of mammary tumors (11, 20), and probably more concentrated in older animals. It may be eliminated by foster nursing on females of strains with a low incidence of mammary tumors. It is active in lyophilized tissue, in a filtrate (Seitz filter), and in desiccated and glycerolated tissue (21). Following ultracentrifugation (110,000 $\times$ *g*) for a period of 60 minutes, the active material appears in traces, if at all, in the fat fraction and in the final supernatant fluid (60). It is probably a colloid of high molecular weight.

The active milk influence may arise *de novo* in animals which have not been in contact with those having a high incidence of mammary cancer (17). If it should thus appear in mice susceptible to the development of spontaneous mammary cancer, the incidence may change from 5 per cent to 98 per cent (17).

III. INHERITED SUSCEPTIBILITY TO SPONTANEOUS MAMMARY CARCINOMA

The data obtained from matings between mice of strains with low and high incidences respectively are in accord with the genetic theory that susceptibility to mammary carcinoma may be transmitted as a single dominant factor (13, 2, 20, 22). The results are somewhat complicated, however, because a higher incidence has been observed in mice of successive litters born to the same mothers (20, 22).

Inherited susceptibility to the development of mammary cancer is not itself altered as a result of foster nursing, and may be inherited through males or females of any genetically susceptible stock.

IV. VARIATIONS IN INCIDENCE OF MAMMARY TUMORS FOLLOWING DIFFERENT EXPERIMENTAL PROCEDURES IN STRAINS OF LOW INCIDENCE

A. Discussion

Inbred strains of mice are to the biologist what pure chemicals are to the chemist. Following a sufficient number of generations of brother-to-sister matings all the mice of an inbred stock are theoretically homozygous as far as their inherited characteristics are concerned. Living material is, however, subject to mutations and some of these may be of such nature as not to become apparent for a number of generations.

It is assumed that every individual of an inbred strain, or subline of an inbred strain, is genetically like every other member of that particular stock. Thus, so far as its genetic susceptibility to the development of spontaneous mammary cancer is concerned, we may suppose that it will be either susceptible or nonsusceptible. Consequently it is probable that any differences observed in the incidence of mammary cancer are a result of different amounts of estrogenic stimulation and/or different concentrations of the active milk influence received while nursing or from forced feeding. The level of estrogenic stimulation may result from intrinsic causes (amount secreted in virgin females) supplemented by extrinsic causes (normal breeding, forced breeding, or injection).

The incidence of mammary tumors in virgin and breeding females of the C57 black strain is less than 1 per cent (37), but when fostered by females of the A stock (high incidence) the *fostered females* of the C57 black stock and *their progeny* had an incidence of 10.6 per cent (15). Andervont observed an incidence of 10.3 per cent in C57 black mice nursed by females of the C3H stock and continued as breeders (1, 6, 50). When animals of the C57 black stock were kept as virgins after having been nursed on females of the dilute brown stock, van Gulik and Korteweg (58) found that 13 per cent, and Murray (47) that 9.2 per cent, developed mammary tumors.

Bagg and his coworkers (8–10) have used mice of the C57 black stock in studies on forced breeding or the functional test. When mice of subline Y were used some developed mammary tumors, whereas none appeared in those of subline A (9). Bagg (9) fostered

18 females of subline A on mice of the C3H stock and found that 50 per cent of the fostered females developed mammary tumors while others were still living without tumors at the time the report was made.[3]

Little and Pearsons (38) also subjected approximately 100 female mice of the C57 black stock to the functional test and did not observe the development of mammary tumors in any of the animals. These experimental animals were all nursed by their mothers.

When females of the C57 black stock which had been nursed by females of the A stock were used to nurse mice of a susceptible stock, a high incidence of tumors was noted in these susceptible animals (15), showing that the milk influence remained active in mice genetically nonsusceptible to spontaneous mammary tumors.

Strong (53, 54) found that breeding females of the CBA strain had an incidence of mammary tumors of approximately 4 per cent. A subline secured from Strong had an incidence of 13.5 per cent in 125 breeding females (12). Suntzeff and his associates (56) found that 5.9 per cent of 202 breeding females and 0.7 per cent of the virgin females of the CBA strain, descended from Bittner's line, had spontaneous mammary tumors.

Mammary tumors have been induced by estrogen injection in males and females of the CBA strain by Gardner (27, 29), using mice of Strong's stock. Mice of the same strain, but of a different subline (12), did not develop tumors in similar studies conducted by Bonser, Stickland, and Connal (23), Bonser and Robson (24), and Suntzeff and his associates (56). Gardner (29) also found that a high incidence of mammary tumors could be produced in females of the CBA stock by forced breeding; but Bagg (8) was unable to obtain any tumors in mice descended from Bittner's line in similar studies.

Breeding females of the A stock descended from a female fostered by a female of the CBA stock (12) had a low incidence of spontaneous mammary tumors (8, 17) and no tumors were induced by estrogens (16, 19). If, however, the members of this fostered subline of the A stock acquire an active milk influence, a high incidence of mammary tumors will result in breeding females (17) and in consequence of the injection of estrogens (19).

Gardner (29) stated that the CBA females used in his experiments transferred to their offspring a tendency to develop mammary tumors in breeding females and after the injection of estrogen. This maternal influence as regards spontaneous or induced mammary tumors was not evident in the mice of Bittner's line of CBA mice or in mice fostered by these females. Since an active milk influence may arise without contact with females from strains of high incidence, by changes in the inactive influence or *de novo* (17), it is possible that such a change occurred in the constitution of the milk influence in the sublines of CBA mice studied by Gardner. Our results following the development of an active milk influence in mice fostered by CBA females were similar to those reported by him.

Andervont (3) observed that approximately 2 per cent of breeding females of the C strain will develop spontaneous mammary tumors. None of the fostered females of the C stock nursed on C3H mice and kept as virgins developed mammary tumors. Those tested by forced breeding had an increasing incidence according to the number of litters born to the mice of the various groups (3). The incidence was higher also in mice subjected to forced breeding and nonsuckling than in those treated with estrogens (50, 51). Andervont (personal communication) found that fostered females of the C stock which died without having developed cancer had progeny with a high incidence. These observations are characteristic for mice of strains with a high tumor incidence, which are considered to carry genetic susceptibility to the development of mammary tumors and which have, in addition, the active milk influence.

B. Comparison of C57 Black, C, and CBA Strains

When mice of the C57 black and C strains were nursed by their mothers (inactive milk influence) a low incidence of mammary tumors was obtained in virgin, breeding, and estrogen-treated females.

When mice of the C57 black stock were nursed by females from different strains of high incidence, the occurrence in the fostered C57 females, virgin and breeding, and the progeny of the latter, was approximately 10 per cent. The incidence was no higher among offspring of the fostered mice that died with cancer than among offspring of the fostered mice that died without having developed cancer. The milk influence was active, however, because when the fostered C57 females were used to nurse mice susceptible to mammary cancer a high incidence was obtained in these susceptible mice. The presence of an active milk influence and the estrogenic stimulation elicited in virgin and normally breeding females were able to overcome the threshold of nonsusceptibility in only 10 per cent of the C57 black mice. If the amount of estrogenic stimulation was increased by forced breeding (or the administration of estrogens), a high incidence of tumors was noted.

[3] Bagg (personal communication) is of the opinion that some of the mice of his C57 black stock, subline Y, developed an active milk influence. Some mice used in these tests were raised by foster mothers (8).

Females of the C stock with inactive milk influence have a low incidence of mammary tumors whether breeding or treated with estrogen. When they have the active milk influence, obtained by fostering them on females of strains with a high rate, virgin females of the C stock have a low incidence of tumors and breeding females a high one. Mice of this stock are probably genetically susceptible to the development of spontaneous mammary tumors, since the fostered mice that died without having developed cancer had offspring with a high incidence. These results are summarized in Table I.

Table I: Incidence of Mammary Tumors in Female Mice of the A, C3H, C, and C57 Black Stock under Different Experimental Conditions

Strain	Milk influence in nursing females	Genetic constitution for spontaneous mammary tumors	Estrogenic stimulation	Incidence of mammary tumors, per cent	Average tumor age, months
A (13)	Active	Susceptible	Virgin females	5	19
A (17)	"	"	Breeding females	96	10
A (17)	Inactive	"	" "	5	17
A (19)	Active	"	Estrone	75	—
			Estradiol benzoate	44	—
A (19)	Inactive	"	Estrone	0	—
			Estradiol benzoate		—
C3H (18)	Active	Susceptible	Breeding females	92	10
C3H (18)	Inactive	"	" "	2	14
C3H (19)	Active	"	Estrone	60	..
C3H (19)	Inactive	"	"	0	..
C3H (4)	Active	"	Virgin females	97	10
C3H (4)	"	"	Breeding females	91	9
C (3)	Inactive	Susceptible	" "	2	—
C (3)	Active	"	Virgin females	0 *	..
C (3)	"	"	Breeding females	75+ *	9 *
C (50, 51)	Inactive	"	Stilbestrol	3	11
C (50, 51)	Active	"	"	70	9
C57 (38)	Inactive	Nonsusceptible	Virgin females	0	—
C57 (38)	"	"	Breeding females	1	21
C57 (15) fostered and progeny	Active	"	" "	11	13
C57 (1, 50)	"	"	" "	10	—
C57 (59)	"	"	Virgin females	13	—
C57 (47)	"	"	" "	9	—
C57, line A (9)	Inactive	"	Forced breeding	0	—
C57, line A (9)	Active	"	" "	50 *	—
C57 (38)	Inactive	"	" "	0	..
C57, male and female (55, 57)	"	"	Injection	1	—
C57 females (57)	Active	"	"	33	—
C57 females	"	"	"	?	..

* Some living without tumors.

Shimkin, Grady, and Andervont (51) have demonstrated that the foster nursing of mice from the insusceptible C strain by mothers of the susceptible C3H stock causes hyperplastic changes in the mammary epithelium.

The genetic constitution of CBA mice for the development of spontaneous mammary tumors has not been determined. Early work with this strain showed that they transferred the inactive milk influence, but more recent experiments by Gardner indicate that mice of the Strong CBA stock may have the active milk influence. Further study of the various sublines of this strain is needed.

V. VARIATIONS IN INCIDENCE OF MAMMARY TUMORS FOLLOWING DIFFERENT EXPERIMENTAL PROCEDURES IN STRAINS OF HIGH INCIDENCE

It is probable that mice of all inbred strains with a high incidence of spontaneous mammary tumors in breeding and/or virgin females have an inherited susceptibility for these tumors. *This inherited susceptibility is not altered or removed as the result of foster nursing on females of strains with a low incidence of mammary tumors* (13, 14).

The following strains of mice with high incidence have been used in foster nursing experiments: A (11), C3H (1, 9, 20), dilute brown (59, 47), and the Paris R III (57). In every experiment it has been demonstrated that the females of these strains transfer the active milk influence to their progeny. Likewise, the

incidence of tumors in these strains may be reduced as the result of foster nursing the young to females of strains with an inactive milk influence. This transfer must take place within a few hours after birth. In only one study have observations been made on the progeny of fostered animals (A stock).

Females of the A strain must be used as breeders before a high incidence of spontaneous mammary tumors will be obtained (14). Thus only breeding females are considered in the following statements.

When the young born to females of the A stock were transferred within a few hours after birth to females of the C57 black or CBA stocks, a low incidence of mammary tumors was observed in the fostered mice. If, however, the fostered females obtained a small amount of the active milk influence from their maternal parents before they were fostered, tumors might result. The progeny of these cancerous fostered mothers gave an incidence which, while lower than that observed in the control stock, was higher than that recorded among the progeny of the noncancerous fostered females. The incidence rose with increasing age of the mothers, confirming the theory (20, 22) that the milk influence is more concentrated in older animals. Other evidence exists (48) for the occurrence of variations in the intensity of the milk influence in different strains, as well as at different ages within the same strain. In the control A stock a high incidence of mammary tumors was observed among the progeny of mothers which died without mammary tumors—suggesting that these mothers might have developed tumors had they lived longer (12).

The progeny of fostered mice that died without having developed cancer had progeny with a low incidence of mammary tumors. If any of these progeny had mammary tumors, their progeny in turn also had a low incidence, unless the cancerous mothers had developed an active milk influence (17, 20).

VI. PRODUCTION OF INDUCED ESTROGENIC MAMMARY TUMORS IN SUSCEPTIBLE AND NON-SUSCEPTIBLE STRAINS

A. Discussion

Gardner (28) and Loeb (41) have published reviews on the development of mammary tumors following the administration of estrogenic hormones. Gardner gave the following summary of the work to 1939:

"The observations on mice with known incidences of tumor appearance indicate that intrinsic factors other than estrogens play a significant role in mammary carcinogenesis. In other strains of mice, however, the influence of these intrinsic factors may be largely dominated by the effects of estrogens administered for long periods. In other strains, characterized by very low incidence of spontaneous carcinogenesis, a few mammary tumors have been obtained by administration of estrogens."

More recent studies have demonstrated that the type of milk influence received also plays a role in the development of induced estrogenic as well as spontaneous mammary tumors (57, 16, 19, 50, 51).

These comparisons are confined to two strains of mice of high incidence and two strains with a low incidence of mammary tumors of known genetic constitution. The strains with a high incidence are: the C3H, with high incidence in virgin and breeding females (4–6), and the A, with low incidence in virgin females and high incidence in breeding females (13, 17). Control mice of these stocks have the active milk influence and inherit the genetic susceptibility for the development of spontaneous mammary tumors. The two strains of low incidence are the C57 black and the C. Mice of both strains probably transfer the inactive milk influence; mice of the C stock are genetically susceptible to the development of spontaneous mammary tumors (3, 50, 51), whereas mice of the C57 black stock are genetically nonsusceptible (13, 22).

Gardner (27) found that a higher incidence of estrogen-induced mammary tumors could be produced in mice of the C3H strain than in animals of the A stock. Loeb and Suntzeff (42) also noticed that the mammary glands of mice of the A strain were more resistant to the effects of estrogens than were those of the C3H mice. Mice of these two strains would have the active milk influence. Since the mice were not used as breeders it is probable that the normal intrinsic production of estrogens was greater in the mice of the C3H stock than those of the A strain. This conclusion is based on the instances observed in the virgin females.

If the mice of these two strains have the inactive milk influence as the result of foster nursing, tumors are rarely produced as a result of estrogen administration (16, 19, 50).

Less than 1 per cent of mice of the C57 black stock have developed mammary tumors after treatment with estrogenic hormones, as determined by Suntzeff and his associates (55, 56), by Haagensen and by Gardner, as reported by Twombly (57), and by Bittner (16, 19), in animals proved to have the inactive milk influence. Twombly (57) fostered mice of the C57 black stock on females of the R III (high incidence) strain and found that 33 per cent of the fostered males developed mammary carcinoma following treatment with estrone.

In Andervont's experiments (3) approximately 2 per cent of breeding females of the C stock developed spontaneous mammary tumors, and about the same incidence was obtained following the injection of estrogenic hormones. When mice of the C stock were nursed by females of the C3H strain, Shimkin and Andervont (50) and Shimkin, Grady, and Andervont

(51) were able to induce estrogenic tumors in 70 per cent of the fostered females and 39 per cent of the fostered males.

B. Summary

Mammary tumors may be induced by estrogen in mice of strains which are genetically susceptible to the development of spontaneous mammary tumors and have the active milk influence. If the mice are genetically susceptible but have the inactive milk influence, induced mammary tumors seldom occur. If genetically nonsusceptible mice have the inactive milk influence few induced mammary tumors will result; if mice genetically nonsusceptible to the development of spontaneous mammary tumors have the active milk influence, the injection of large amounts of estrogenic hormones will overcome the threshold of nonsusceptibility and induced mammary tumors will arise.

VII. Possible Types of Mammary Tumors as Determined by the Incidence in the Progeny of Cancerous and Noncancerous Mothers

The incidence of mammary tumors in the progeny of cancerous and noncancerous mothers of inbred strains suggests that there are at least two types of these neoplasms in mice (14).

The difference can be recognized only by observing the progeny of the individual mice under investigation. As they must be used for breeders, it may be assumed that estrogenic stimulation of the mammary tissue has taken place. Differences in the etiology of these two types of mammary tumors must result from variations in the genetic susceptibility or nonsusceptibility of the mice or in the nature of the milk influence.

In strains of inbred mice with a high incidence. it is of small moment whether or not the females develop mammary tumors—the offspring of all females will have a high incidence. All such mice would be genetically susceptible to spontaneous mammary tumors and would have the active milk influence. This class of mammary tumors, the "inherited" type, requires that all the etiological agents be present and active.

The "noninherited" variety appears in animals which are genetically susceptible and have the inactive milk influence, or in those which are genetically nonsusceptible and have either the active or the inactive milk influence. In these cases the incidence of mammary cancer among the progeny of cancerous mothers is usually the same as that observed in the progeny of noncancerous mothers. These incidences are generally characteristic of the entire strain.

It has been demonstrated that genetically nonsusceptible mice nursed by females of strains with a high incidence of mammary tumors actually do have the active milk influence, because when they are used to nurse susceptible mice a high incidence results in the fostered susceptible animals (15). The active milk influence and the amount of estrogenic stimulation produced in virgin or breeding females was able to overcome the threshold of genetic nonsusceptibility of the mammary glands in only a few animals. If the amount of estrogenic stimulation is increased by forced breeding or injection, a larger percentage of fostered genetically nonsusceptible animals develop tumors.

VIII. Possible Role of Each "Influence" in the Etiology of Mammary Cancer

Since 1939 (14) three influences have been recognized in the etiology of the inherited type of mammary tumors in mice. May one of these be the "inciting influence" which causes the normal gland to become cancerous, or should they all be considered as "inciting influences"?

A summary of the results obtained under different experimental conditions in four strains of mice is given in Table I. Most of the observations were made on females but in a few cases it has been necessary to use the data obtained from males. Two of the strains had a high incidence and the others a low one when nursed by their maternal parents of the control stocks.

In experiments made to determine the role of each influence, the genetic constitution for inherited susceptibility or resistance to spontaneous tumors would probably remain constant during the life of the animals. The concentration of the active milk influence (20, 22) increases with the age of the females, particularly in mice which are used as breeders. It is likely, too, that if estrogenic hormones are secreted, causing development of the mammary tissue, the level of estrogenic stimulation also will increase as the mice of reproductive age become older.

If mice which are genetically susceptible (A) or genetically nonsusceptible (C57 black) are nursed by females of a strain with a high incidence of mammary tumors (active milk influence), they should obtain approximately the same amount of the milk influence and the concentration should remain the same in those of comparable ages.[4] Furthermore, there is little reason to expect that the amount of estrogenic hormones secreted as the result of normal breeding would differ greatly in the females of these two strains. Thus it is probable that the concentration of the active milk influence and the estrogenic stimulation would be as

[4] When nonsusceptible animals with the active milk influence were used to nurse susceptible animals, a high incidence of mammary tumors was observed in the susceptible mice (15).

nearly alike as is possible of attainment under experimental conditions. The chief variable would be genetic. In the genetically susceptible animals the incidence was 96 per cent (17) and in the genetically nonsusceptible mice 11 per cent (15). This difference must result directly or indirectly from variations in the genetic constitution of the susceptible and nonsusceptible animals. Whether or not the genetic susceptibility is "the mammary tumor inciter" in these animals cannot be determined. If the amount of estrogenic stimulation in the nonsusceptible animals was increased by forced breeding, the incidence was 50 per cent (9), and males of the C57 black strain with the active milk influence had an incidence of 33 per cent following the administration of estrogens (57).

While it is known that mammary carcinoma can arise only in a gland which has undergone a certain degree of development, it has not been determined whether the hormones merely prepare the gland for its initiation by some other influence or influences or act as a direct cause (36).

Since the initial stimulus resulting from the estrogenic hormones may take place months before a mammary tumor appears, it would seem that the hormones only prepare or condition the glands for the malignant change. An increase in the amount of estrogenic stimulation will prepare the glands for this transformation in a shorter period and indirectly increase the incidence of tumors, and the same results may be obtained by feeding mice various amounts of milk containing the active milk influence (16).

The architecture of the mammary glands of mice which have received the active milk influence differs from that seen in mice which have received the inactive milk influence (59, 51).

If mice which are susceptible to the development of spontaneous mammary tumors are permitted to have two or three litters before they are given milk containing the active milk influence, few tumors result; but if mice of the same stock are given milk with the active influence when they are 4 to 6 weeks of age, mammary tumors will develop in a high percentage, depending upon the amount and concentration of the active influence (16). The amount determines also the average tumor age. When milk containing the active influence is fed to adult females with mammary glands developed on the basis of an architecture resulting from an inactive milk influence, the active influence may be unable to take part in the development of mammary cancer. In fact it cannot do so unless it is able to affect the glands as soon as they start to develop. Thus the estrogenic hormones and the active milk influence would play similar roles in the etiology of mammary cancer in mice. Finally, the inherited susceptibility or nonsusceptibility to spontaneous mammary cancer must be considered as a determinant of the structure of the mammary glands (59).

Thus each of three influences in the etiology of the inherited type of mammary cancer in mice may be considered as a "mammary tumor preparatory influence or inciter." If one influence is not present or is inactive, the noninherited type of mammary cancer generally results. It is more likely, however, that the milk influence, genetic susceptibility, and estrogenic stimulation should be considered as complementary the one to the other and, as such, they might be called the "mammary tumor milk inciter (MTMI)," the "mammary tumor inherited inciter (MTII)" or "mammary tumor susceptibility inciter (MTSI)," and the "mammary tumor estrogenic inciter (MTEI)."

These symbols are useful only as abbreviations for the various inciters. It would lead to confusion if they were used to describe the concentration of the MTMI or the amount of stimulation produced by the MTEI resulting from intrinsic or extrinsic causes.

IX. CONCLUSIONS

For the development of mammary carcinoma in virgin and breeding females three agents usually must be present and active:

(a) An active mammary tumor milk influence which is generally transferred by nursing.

(b) Hormonal stimulation of the mammary tissue resulting in growth suitable for the cancerous change.

(c) An inherited susceptibility to the development of spontaneous mammary tumors which may be transmitted by males and females of the susceptible stock.

The active milk influence may be greater in some strains than in others, and may be more concentrated in individual females after they have given birth to three or more litters. The amount of the active milk influence that mice obtain may determine the incidence of mammary tumors, the average tumor age, and the incidence of mammary tumors observed in the progeny of experimental animals.

The active milk influence may originate following changes in the "inactive" milk influence, or *de novo*. It remains active in lyophilized tumors, in glycerol-treated and desiccated tissue, and in a filtrate of material containing it.

In association with intrinsic factors the active milk influence may be instrumental in determining the architecture of the mammary glands.

The amount of estrogenic hormones secreted by virgin females of various strains of mice may be determined by intrinsic factors, and is not causally related to the development of mammary cancer. The amount

secreted by virgin females of some strains is evidently sufficient to result in neoplasms provided the other necessary stimuli are present and active. In mice of other strains the amount resulting from intrinsic causes must be supplemented by that furnished through such extrinsic causes as breeding or injection before many mammary tumors will develop.

A restricted diet may lower, and a diet rich in protein may increase, the amount of hormonal secretion.

Mice of strains with a low incidence of mammary tumors in breeding females may have:

(a) An inactive milk influence and be susceptible or nonsusceptible to the development of spontaneous mammary tumors.

(b) An active milk influence and be nonsusceptible to the development of spontaneous mammary carcinoma. If this active milk influence is transferred to genetically susceptible mice by nursing a high incidence of tumors will result.

Mice of one subline of a nonsusceptible strain may have the inactive milk influence, and those of another subline of the same strain may have the active milk influence. Only by testing can this difference be demonstrated.

The administration of estrogenic hormones will produce mammary tumors in males and females from strains of mice with the active milk influence. The induced incidence is usually higher in females than in males.

The stimulation produced by large amounts of estrogenic hormones may overcome the threshold of genetic nonsusceptibility in the presence of an active milk influence.

The incidence of induced estrogenic tumors is higher in mice that are genetically susceptible to the production of mammary tumors.

Few induced tumors have been elicited in mice that were genetically susceptible or nonsusceptible to mammary tumors and that had the inactive milk influence. The increased estrogenic stimulation was unable to overcome the threshold resulting from the inactive milk influence in susceptible or nonsusceptible tissue.

In the same strain of mice forced breeding with nonsuckling (the functional test) has produced a higher incidence of mammary tumors than has the administration of estrogenic hormones. Mammary tumors have not been observed to develop in many force-bred mice which did not have the active milk influence.

Two or more types of mammary carcinoma may develop in mice. These may be termed the inherited and noninherited types.

The noninherited mammary tumors may arise in mice that have the inactive milk influence and are susceptible or nonsusceptible, or in those that have the active milk influence and are nonsusceptible. The incidence of mammary tumors among the progeny of these cancerous females is the same as that characteristic of the strain. Many, if not all, of these tumors may be described as developing by a process analogous to somatic mutation in that the progeny of their bearers have not a high incidence.

The inherited type of mammary tumors develops in mice of strains that have the inherited susceptibility and the other active influences. The progeny of both cancerous and noncancerous females of these strains usually have a high incidence.

It is possible that mammary tumors produced by estrogenic hormones in nonsusceptible tissues may be grouped with the noninherited mammary tumors, and those produced in susceptible tissue with the inherited.

Only by observing the incidence of mammary tumors in the progeny of cancerous females is it possible to determine, in many instances, the type which an animal may have.

The influences underlying the development of mammary tumors in mice may each be considered as a "mammary tumor preparatory influence" or a "mammary tumor inciter."

If they are called inciters, a distinction should be made between the different varieties as: the mammary tumor milk inciter (MTMI); the mammary tumor inherited inciter (MTII) or the mammary tumor susceptibility inciter (MTSI); and the mammary tumor estrogenic inciter (MTEI).

These symbols are useful only as abbreviations and could not be used to describe the concentration of the MTMI or the amount of stimulation of the MTEI resulting from intrinsic and/or extrinsic causes.

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Endocrine-Cancer Conference

Hotel Traymore, Atlantic City

June 5-6, 1942

Program

I. Friday, 2:00 p.m.—HORMONAL FACTORS IN THE INDUCTION OF TUMORS.

1. W. U. Gardner. Mammary Tumors in Mice Receiving Sex Hormones.
2. Michael B. Shimkin and Howard B. Andervont. Effect of Foster Nursing on Induction of Mammary Tumors in Mice with Estrogens.
3. Edgar Allen. Cancer of the Cervix Uteri after Estrogenic Treatment.
4. Luis Vargas. The Tumorigenic Action of Steroid Hormones with Emphasis on Uterine Fibroids.
5. Warren O. Nelson. Some Effects of Hypophyseal Tumors Induced by Treatment with Estrogens.
6. Charles W. Hooker. Testicular Tumors in A Strain Mice after Estrogenic Treatment.
7. Michael B. Shimkin and Hugh G. Grady. Interstitial Cell Tumors of Testes Induced with Stilbestrol in Mice.
8. F. X. Paletta. The Influence of Estrogen on Experimental Epidermal Methylcholanthrene Carcinogenesis in Mice.
9. W. U. Gardner. Lymphoid Tumors in Estrogen-Treated Mice.

II. Friday, 8:00 p.m.—INTRINSIC FACTORS AND EXPERIMENTAL PROCEDURES INFLUENCING ORIGIN AND GROWTH OF TUMORS.

10. H. S. N. Greene. Mammary and Uterine Tumors in Rabbits.
11. Carroll A. Pfeiffer. Experimentally Induced Endocrine Imbalance and Atypical Genital Conditions in Mice.
12. John J. Bittner. Possible Relationship of the Estrogenic Hormones, Genetic Susceptibility, and Milk Influence in the Production of Mammary Cancer in Mice.
13. George W. Woolley. Adrenal Cortical Tumors and Their Syndromes in Mice.
14. William Cramer. Morphological Changes in the Mouse Adrenal and Their Functional Significance, with Special Reference to the Hormonal Etiology of Breast Cancer.
15. James B. Hamilton. Relation of the Incidence of Testicular Tumors to Cryptorchidism.
16. A. W. Wright and J. M. Wolfe. The Effects of Prolonged Estrogen Administration on Spontaneous Mammary Fibroadenomas in the Albany Strain Rats.
17. Jacob Heiman. Comparative Effects of Estrogen, Testosterone, and Progesterone on Benign Mammary Tumors of the Rat.
18. Alexander Lipschütz. Endocrine Aspects of Antitumoral Autodefense.

III. Saturday, 9:00 a.m.—TUMORS AFFECTING SECRETION AND EXCRETION OF HORMONES.

19. Charles Huggins. Endocrine Relationships of Prostatic Cancer.
20. J. P. Pratt. Sex Precocity, Virilism, and Adrenal Cortical Tumor.
21. G. H. Twombly. Excretion of Hormones by Patients Suffering from Testicular Tumors.
22. Howard C. Taylor, Jr. Excretion of Estrogens and Androgens in Breast Carcinoma in Varying Conditions of Hormone Administration.
23. Ira T. Nathanson. Sex Hormone Excretion in Neoplastic Disease.
24. L. Strait, E. McCawley, and I. Perry. Spectroscopic Study of Triphenylethylene in the Normal and Cancerous Tissues of Mice Treated with Triphenylethylene.
25. K. Dobriner. The Excretion of 17-Ketosteroids by Normal and Diseased Persons.
26. Gregory Pincus. Steroid Excretion in Cancerous and Noncancerous Persons.

Abstracts

3. CANCER OF THE CERVIX UTERI AFTER ESTROGENIC TREATMENT. EDGAR ALLEN.* (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Long-continued treatment of mice with high doses of estrogenic hormone is followed by lesions of the cervix which grade from early anaplastic conditions, through tumors of increasing size, to large squamous cell carcinomas. The largest tumors may almost completely fill the pelvis and sometimes prolapse from the vagina. Those listed as stage IV frequently invaded muscular tissue of the rectum and urinary system.

The most frequent site of the lesion is at the posterior lip of the cervix but there may be multiple lesions.

In 2 groups of hybrids between the C57 and the CBA strains an incidence of 50 and 62 per cent of tumors was obtained in animals which survived estrogenic treatment for more than 1 year.

That this cancerous tissue has attained the capacity for autonomous growth is shown by successful transplants to other animals of the same strain, where the tumor continues to grow in both males and females without continued injections of estrogenic hormone into the hosts.

Neither genetic factors nor the milk factor, so important in the incidence of mammary cancer, seems to have much bearing upon the incidence of cervical cancer in these experimental mice. However, among the animals nursed by mothers from strains susceptible to mammary cancer, a few of the hybrids develop mammary cancer at an earlier age than experimental cervical cancer.

Since cervical carcinoma does not appear spontaneously in mice, the high incidence in animals on treatment with estrogenic hormone seems to indicate that endocrine factors may be extremely important in this type of cancer.

The report by Papanicolaou and Traut that cervical cancer in women can be diagnosed by the vaginal smear technic has led us to test this point in experimental cervical cancer in mice. We find it possible in a certain percentage of cases to diagnose cervical lesions, the diagnosis being checked by study later of serial sections. So far we are unable to diagnose cervical lesions in early stages, but stages III and IV can be diagnosed without biopsy. Further tests of this promising diagnostic method are under way.

As yet we have not studied possible retrogression of established lesions after cessation of hormonal stimulation. The exact moment of the transition from the nonmalignant atypical lesion to the malignant neoplasm remains to be found.

* In collaboration with W. U. Gardner.

12. POSSIBLE RELATIONSHIP OF THE ESTROGENIC HORMONES, GENETIC SUSCEPTIBILITY, AND MILK INFLUENCE IN THE PRODUCTION OF MAMMARY CANCER IN MICE. JOHN J. BITTNER. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

The role of estrogenic hormones in mammary carcinogenesis is considered in relationship to the genetic constitution of the mice and the type of milk influence the animals receive while nursing.

In some strains of mice the estrogenic stimulation produced by intrinsic factors in virgin females is sufficient to cause the growth of mammary tissue and result in the development of mammary cancer when the other etiological inciters are active; in other strains, extrinsic causes of mammary estrogenic stimulation (normal breeding, forced breeding, or injection of estrogens) are needed before many tumors will result.

The concentration of the active milk influence may differ in various strains of cancerous mice and be more concentrated in the milk after the females have had 3 or more litters. The amount and concentration of the active milk influence obtained by the animals may also determine the incidence and average age when the other influences are active.

Growth of the mammary tissue is dependent upon the action of the hormones; the architecture which it assumes may be determined by the nature of the milk influence that the mice obtain.

High cancer strains may be made low by giving newborn young to females having the inactive milk influence. Foster nursing does not change the susceptibility or resistance of mice for the development of mammary cancer. An active milk influence may arise in mice which have not been in contact with animals of high cancer strains.

By observations on the incidence of mammary tumors developing in the progeny of cancerous and noncancerous mothers it is possible to differentiate at least 2 types of mammary neoplasms.

An excessive amount of estrogenic stimulation (forced breeding or injection) may overcome the threshold of nonsusceptibility of the mammary tissue in the presence of an active milk influence. Few mammary tumors have been produced following the same treatment in mice which were susceptible or nonsusceptible to the development of spontaneous mammary tumors if the milk influence was inactive.

Mice of strains with a low incidence may have the active milk influence and be nonsusceptible, or have the inactive milk influence and be susceptible or nonsusceptible for the development of spontaneous mammary tumors. A low incidence is usually observed in the progeny of cancerous and noncancerous females. If mice of low cancer strains are susceptible and are nursed by females of high cancer strains, the fostered mice and their progeny will have a high incidence. The progeny of cancerous and noncancerous females of a susceptible stock, having the active milk influence, will have a high incidence of mammary tumors.

14. MORPHOLOGICAL CHANGES IN THE MOUSE ADRENAL AND THEIR FUNCTIONAL SIGNIFICANCE, WITH SPECIAL REFERENCE TO THE HORMONAL ETIOLOGY OF BREAST CANCER. WILLIAM CRAMER. (The Barnard Free Skin and Cancer Hospital, St. Louis, Mo.)

Since Cramer and Horning in 1936 suggested the possibility that the adrenal gland is concerned in the hormonal

etiology of breast cancer in mice a number of facts have come to light to confirm their conception. These are:

1. Removal of the gonads in male and female mice of high cancer strains is followed by a nodular hyperplasia of the peripheral part of the cortex and by a development of the mamma and, subsequently, carcinogenesis in the mamma in both males and females (Woolley, Fekete, and Little).

2. Removal of the adrenals impairs the functional effects of the estrogenic ovarian hormone on the mamma and the other organs normally reactive to the hormone.

3. Estrinization protects against the loss of the adrenals.

These facts demonstrate the existence of a synergism between the adrenal cortex and the gonads, especially the ovary. There is no evidence for the existence of a similar synergism between the adrenal medulla and the gonads. The mouse adrenal is subject to a variety of morphological changes peculiar to that gland which may develop either spontaneously or in response to alterations in the functional activity of the gonads. They affect different parts of the gland and their functional significance varies with the part affected. These changes are:

1. Nodular hyperplasia of the peripheral part of the cortex visible to the naked eye.

2. Hyperplasia of the cortex in the juxtamedullary portion (X-zone).

3. Brown degeneration limited to the juxtamedullary portion of the cortex.

4. Brown degeneration affecting mainly the medulla.

5. Changes in volume of the medulla occurring spontaneously in females and induced experimentally by the injection of testosterone (increase in volume) or by castration (diminution in volume—X-zone).

As a result of these morphological changes the functionally effective volume ("f.e.v.") of the cortex or of the medulla may be either separately increased or separately diminished, or an increase in the f.e.v. in one constituent part of the gland may be associated with a decrease in the f.e.v. of the other constituent part. The presence of these morphological changes indicates the existence of an endocrine imbalance which may have its origin in the adrenal itself, or in the gonads, or in some other endocrine organ.

A summary of the observations on these changes recorded so far in the literature shows that those morphological changes which either increase the f.e.v. of the cortex, or diminish the f.e.v. of the medulla, or induce a combination of both, are associated with a high incidence of breast cancer. Morphological changes which induce opposite effects are associated with a low incidence of breast cancer. The same condition in the mamma can, therefore, be associated with a variety of morphological changes in the adrenal.

It is not suggested that any of these morphological changes are the direct cause of cancer of the breast. They are probably mainly concerned with the degree of epithelial proliferation in the mamma, on the basis of which cancer develops if the milk factor is present.

These conclusions have an application to the etiology of that type of breast cancer in the human subject which is due to an endocrine imbalance. In women breast cancer due to hormonal factors is most likely to be found in patients with a family history of breast cancer, or in patients in which cancer has developed in both breasts. Analysis of the hormonal etiology of breast cancer in the mouse suggests in such cases a search for adrenal abnormalities indicative of the existence of an endocrine imbalance, such as, for instance, cortical adenomata.

25. THE EXCRETION OF 17-KETOSTEROIDS BY NORMAL AND DISEASED PERSONS. K. DOBRINER. (Memorial Hospital, New York, N. Y.)

A systematic investigation has been initiated of the amounts and varieties of steroid hormones excreted by normal persons and patients with cancer. The studies have been collaborative between the Memorial Hospital group and Dr. Fieser and Dr. Lieberman of Harvard University.

Two to 6 months' collections of individual urines from 6 normal persons and from patients with cancer have been examined. The urines were hydrolyzed with sulfuric acid. The ether-soluble material was partitioned into the acidic, phenolic, and neutral fractions. The neutral material was separated with Girard's reagent (T) into ketonic and nonketonic fractions. The ketonic material was further processed with digitonin into the 3-alpha-hydroxy- and 3-beta-hydroxy-ketosteroid fractions.

The results show that cancer patients excrete in general smaller total amounts of ketosteroids than do normal subjects. The 3-beta-hydroxy-ketosteroid fraction in the normal persons was 0.6 to 2.2 per cent of the total ketosteroids. Five patients with cancer showed the same range except for one with cancer of the prostate who excreted 6 per cent of the total 17-ketosteroids as beta material.

By a systematic chromatographic adsorption analysis of the material not precipitated by digitonin, 4 main fractions were obtained. In those from the 6 normal persons, fractions I and IV each consisted of 5 to 15 per cent of the total ketosteroids. Fractions II and III together made up 70 to 90 per cent of the total and were found to be present in the ratio 4:5. The distribution of the material was very uniform. In the fractions from the patients with cancer the distribution was not as consistent as that found in normal persons. Fractions I and IV were, with one exception, larger than normal, and the ratios between fractions II and III were irregular.

From fraction I in 4 of the materials from the normal subjects both $\Delta^{3,5}$-androstadienone-17 and androstenone-17 were isolated and identified. Neither substance was found in the fractions from any of the cancer patients. From fraction II of all 6 normal subjects and 3 cancer patients androsterone was isolated, but it was not found in 3 of the cancer patients. In these 3 cases, ketonic material was present which did not contain androsterone. Fraction III consisted of 3-alpha-hydroxyetiocholanone-17 and was isolated from the material from the 6 normal persons

and from the 6 cancer patients. Fraction IV, which is present in increased amounts in the material from cancer patients, contains a number of substances which have not been described as yet in urines. Work on the identification of those substances is in progress.

Qualitative and quantitative disturbances of the excretion of 3-alpha-hydroxy-ketosteroids and 17-ketosteroids have been found in cancer patients. However, these findings cannot be interpreted to have any specific relationship to cancer until the steroid excretion is investigated in other diseases.

1. MAMMARY TUMORS IN MICE RECEIVING SEX HORMONES. W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Mammary tumors appear in male mice of suitable strains given any of the different estrogenic chemicals in adequate amounts. The suitable strains are those in which the multiparous females develop mammary tumors. Mammary neoplasms do not appear frequently among estrogen-treated male or female mice of strains in which very few spontaneous tumors appear.

In hybrid mice obtained by mating A × C57, C3H × C57, or CBA × C57 the incidence of mammary tumors depended upon whether or not the female parent was from the tumor-susceptible strain. The tendency to develop hypophyseal and testicular tumors, on the other hand, was transmitted by both parents.

Differences in the general morphology of the mammary glands of mice of the different strains or hybrid groups could not be associated with the tendency to acquire mammary tumors in either control or estrogen-injected groups. The presence of localized hyperplastic nodules or diffuse overgrowths of glandular mammary tissue was always associated with the tendency to develop mammary tumors and may represent early stages of the process of cancer formation. At the time these nodules appear the remainder of the breast tissue may be atrophic.

The simultaneous administration of testosterone propionate and estrogens results in a notable reduction of the incidence of mammary tumors and a limitation of mammary growth.

Estrogens may induce some precancerous changes in the breast tissue but there is no proof that they have any effect on the induction of mammary malignancy.

9. LYMPHOID TUMORS IN ESTROGEN-TREATED MICE. W. U. GARDNER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

The title does not imply that lymphoid tumors are induced by estrogens, but merely that lymphoid tumors appear among estrogen-treated mice at a much higher incidence than among untreated control mice of similar origin. When large and highly effective doses of estrogens such as pellets of estrone implanted subcutaneously, or weekly injections of 25 to 50 μgm. of estradiol dipropionate, were administered to mice of the CBA and C3H strains more lymphoid tumors appeared than mammary tumors. Of 303 mice treated, 76, or 25 per cent, died with lymphoid tumors. Among 482 untreated or non-estrogen-treated mice of the same 2 strains, 9 (less than 2 per cent) lymphoid tumors appeared, and 5 of these were among a group of 108 virgin females which lived to be extremely old.

Almost all the lymphoid tumors involved the mediastinum and invaded the lungs, heart, and thoracic wall. Others showed, in addition, a generalized involvement of all lymphoid and myeloid tissues. They always grew when transplanted. None of the untreated mice had a mediastinal tumor without generalized lymphomatosis.

A regression of the thymus and probably other lymphoid tissues occurs when estrogens are injected. Lymphoid tumors apparently arise in these damaged tissues.

10. MAMMARY AND UTERINE TUMORS IN RABBITS. H. S. N. GREENE. (Department of Pathology, Yale University School of Medicine, New Haven, Conn.)

The endocrine organs of rabbits bearing spontaneous uterine and mammary tumors show changes similar to those found in animals subjected to continued treatment with estrogenic substances. The absence of indicative ovarian changes together with a history of recovery from eclampsia and persistent liver damage suggested that an inability of the liver to inactivate a normal secretion of estrone, rather than a primary ovarian hypersecretion, formed the basis of the increased concentration in the circulation.

Evidence that the abnormal constitutional status incident to the alteration in estrin metabolism was essential to the continued growth and development of the tumors was obtained from transplantation experiments. It was found that during dependent or preautonomous stages of development, the tumors could not be transplanted to normal animals. In contrast they were readily transplanted to estrinized animals and in this situs not only grew but continued their development to cancer.

15. RELATION OF THE INCIDENCE OF TESTICULAR TUMORS TO CRYPTORCHIDISM. JAMES B. HAMILTON.* (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Correlation of testicular cancer with ectopy is shown by the facts that: (1) concomitant cryptorchidism existed in 11 per cent of recorded cases of testicular cancer, an association 48 times higher than expected in chance association; (2) in unilaterally cryptorchid men with cancer of one testis, the tumor was in the undescended testis in 97.5 per cent of the cases; and (3) in men with cancer of one testis the possibility of a tumor developing in the other testis is about 25 times greater if the testes have not descended.

Congenital malformation and the endocrine status of the patient are significant in carcinogenesis in the testes, but there is no proof that residence of the testis in an ectopic position is *per se* necessarily an incitant to neoplastic growth.

The subject of testicular cancer allows analysis of a tendency to development of cancer in more than one site. In men with one testicular tumor the likelihood of the appearance of apparently primary cancer in the second testis is several thousand times greater than could be accredited to chance. If the other testis is retained in the abdomen it is all the more prone to the development of cancer. Despite the rarity of bilateral abdominal cryptorchidism, persons with this condition account for one-eighth of all known cases of bilateral testicular cancer.

No particular type of tumor is observed with special frequency in cancer of the undescended testis or in bilateral cases.

Management of the patient with cancer of one testis must take into account the possibility that cancer may appear subsequently in the other testis, particularly if the testis is undescended. The pertinence of the relation of cryptorchidism and testicular cancer is shown in that despite the usual rapidly fatal course of testicular cancer, a tumor has appeared in the other testis in 30 per cent of all such cases with abdominally retained testes.

* In collaboration with Judson Gilbert.

17. COMPARATIVE EFFECTS OF ESTROGEN, TESTOSTERONE, AND PROGESTERONE ON BENIGN MAMMARY TUMORS OF THE RAT. JACOB HEIMAN. (Department of Cancer Research, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

The effects of estrogens and androgens on the growth of benign fibroadenoma have been reported. The present record deals with the effects on similar tumors of progesterone and compares the effects of estrogen, androgen, and progesterone, injected singly or in combination. An attempt was made to duplicate on tumors the effect of progesterone acting synergistically with estrogen on either the duct or alveolar systems, or both, of the mammary gland in early pregnancy.

Intact or castrated male and female rats with spontaneous benign tumors or with auto- or homotransplants were injected with progesterone. Some were estrinized previously, some simultaneously, and some only progesterinized. Two pregnant rats with benign tumors were also progesterinized. Total dosage varied between 9 and 18 mgm. of progesterone and 1 to 2.5 mgm. of estrogen. Sixty rats were used, including 4 with spontaneous tumors and 2 pregnant ones.

Before estrinization or progesterinization 4 Wistar laboratory animals with spontaneous tumors were inoculated with autotransplants. This gave an opportunity to study 3 to 4 growing or receding tumors in one rat with similar tumor morphology. In these 4 rats there were available 12 autotransplants of the first generation, 6 autotransplants of the second generation, and 2 autotransplants of the third generation. In this way the aging of the tumor was slowed, and observation could be carried out on small tumors instead of bulky ones.

Autotransplants were put into 4 and homotransplants into 56 Wistar animals, divided into the following series:

1. Four females with spontaneous tumors.
2. Thirty-eight normal females (2 pregnancies).
3. Six castrated females and 6 normal males.
4. Six castrated males.

1. Progesterone inhibited the growth of the adenomatous portion of spontaneous mammary fibroadenoma of the rat. Shrinkage was followed by fibrosis.

2. Progesterone reduced the percentage of takes of auto- and homotransplants (22 per cent).

3. Eighteen mgm. of progesterone did not interfere with the stimulating effect of 2.5 mgm. of estrogen. Eighteen mgm. of progesterone inhibited the stimulating effect of 1 mgm. of estrogen.

4. Progesterone alone did not affect fibromas growing in castrated females or normal males.

5. Progesterone alone inhibited growth of the glandular portion of adenofibromas in castrated males.

6. Progesterone and testosterone in combination were effective in reducing the percentage of takes to 8.3 per cent, and in inhibiting the growth of the glandular fraction of these tumors.

7. Large doses of progesterone or testosterone are necessary to neutralize the stimulating effect of estrogen on growing fibroadenoma.

8. Rat fibroma, myxoma, and sarcoma are not inhibited by progesterone.

9. Progesterone did not hinder the growth of fibroadenoma in pregnant rats.

10. The action of progesterone on benign mammary fibroadenoma is unlike the progestational effect on the uterus and normal mammary gland.

6. TESTICULAR TUMORS IN A STRAIN MICE AFTER ESTROGENIC TREATMENT. CHARLES W. HOOKER.* (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Thirty-nine of 56 surviving mice of the A strain given weekly injections of 16.6 or 50 µgm. of estradiol benzoate or 0.25 mgm. of stilbestrol had unilateral or bilateral interstitial cell tumors of the testis after 8 months or more of treatment. The tunica albuginea was invaded in 3 instances, and the renal, lumbar, and mediastinal lymph nodes were infiltrated to various degrees in 29 of the tumor-bearing animals. The seminal vesicles and prostate glands indicated production of androgen by 35 of the tumor-bearing animals. Attempts to transplant 5 of the tumors to untreated hosts of the same strain were unsuccessful. In mice that were given estrogen 3 of the tumors grew when transplanted.

The events in the testes culminating in the formation of tumors appear to be: First an enlargement of the Leydig cells and later the appearance of brown cells in the intertubular spaces. Then follows the disappearance of the Leydig cells, apparently to a considerable extent because of their phagocytosis by the brown cells. New Leydig cells then appear to arise by metamorphosis of

mesenchymal or fibroblastic elements. These new cells develop most rapidly in localized areas, forming one or more nodules of interstitial cells. Growth of the nodules accounted for the gross enlargement of the testis. There appear to be 3 generations of tumor cells: The first is large, vacuolated, and strongly eosinophilic; the second resembles the normal Leydig cell; the third is a small, hyperchromatic cell with frequent mitosis. Tumors were composed of any one generation or of combinations of 2 or all 3 generations of cells.

In 15 mice of the A strain receiving weekly injections of 1.25 mgm. of testosterone propionate in addition to 50 μgm. of estradiol benzoate the appearance of testicular tumors was not prevented, but the pace of events was retarded.

Daily injections for 7 to 12 months of 20 rat units of pregnant mare serum gave rise to small nodules of Leydig cells; but further change was not produced. After 6 months of treatment with 0.25 mgm. stilbestrol weekly the testes recovered when treatment was discontinued. Substitution of 20 rat units of pregnant mare serum upon discontinuing stilbestrol after 6 months in 5 animals led to the development of interstitial cell tumors in 2 cases.

* In collaboration with C. A. Pfeiffer.

19. ENDOCRINE RELATIONSHIPS OF PROSTATIC CANCER. CHARLES HUGGINS. (Department of Surgery, The University of Chicago, Chicago, Ill.)

It has been demonstrated in this laboratory that cancer of the prostate in man in many cases resembles adult prostatic epithelium from a functional standpoint, in that it is activated by injection of androgen (testosterone) and inactivated by decreasing the amount of androgen through castration or by elimination of the physiological characteristics of the androgen through estrogenic administration. The amount of inactivation that occurs following castration or estrogen administration is of an order which is clinically useful.

The course of this disease, and the influence of sex hormones upon it, may be studied most objectively and simply by frequent serial observations of the acid and alkaline phosphatases in the serum of patients in whom these enzymes are increased in amount.

In about 60 per cent of the patients with far advanced and metastatic cancer of the prostate a satisfactory response occurs after orchiectomy in that there is a great relief of pain, an increase in appetite and weight, and disappearance of all or nearly all the clinical and roentgenologic evidence of the disease. This improvement has been sustained for as long as 3 years—the maximum period through which these patients have been observed.

In about 30 per cent of the patients, the response is not sustained and within one year clinical symptoms reappear. In about 10 per cent of the cases or less, small clinical improvement results. However, there is evidence that the disease has been influenced through hormonal modifications even in this failure group. Usually the serum phosphatases decrease in amount, although they do not reach normal levels. Moreover, a peculiar phenomenon has been observed in certain of these patients in that the primary neoplasm has undergone atrophy while the metastases have continued to grow. Estrogen does not importantly supplement orchiectomy as a therapeutic agent in this failure group.

Functionally there are two types of prostatic cancer, judging from their response to endocrine modification: adenocarcinoma, in which the response is good; undifferentiated carcinoma, where the response is less striking and less sustained.

18. ENDOCRINE ASPECTS OF ANTITUMORAL AUTODEFENSE. ALEXANDER LIPSCHÜTZ. (Department of Experimental Medicine, National Health Service, Santiago de Chile)

Uterine and extragenital abdominal fibroids induced by estrogens in the guinea pig can be employed for studying experimentally the means by which the organism is able to counterbalance the tumorigenic action of steroids. Five different lines of approach were tried:

1. The sexual rhythm as an antitumorigenic factor.—Abdominal fibroids were induced when 3 injections of 20 μgm. of estradiol benzoate were administered weekly during 3 months. But when the same number of injections were given over a period of 12 months, each week of injection being followed by 2 weeks free of injections, there were no fibroids.

2. Differential fibromatogenic activity of various natural estrogens.—Subcutaneously implanted pellets of urinary estrogens (estriol and equilenin) were found to be less fibromatogenic than those of ovarian estrogens (estradiol and estrone).

3. The inactivation of estrogens in the liver.—When pellets of esterified estradiol were implanted into the spleen, abdominal fibroids failed to appear even when the estrogenic activity was maintained for 2 months. When pellets of estrogens were implanted into the liver the frequency of fibroids dropped greatly (83 per 100 with subcutaneous pellets and only 32 per 100 with intrahepatic ones).

4. Adjustment of the steroid balance.—The fibromatogenic action of estrogens was prevented by the simultaneous administration of different steroids (progesterone, desoxycorticosterone, dehydrocorticosterone, testosterone).

5. The bearing of molecular structure of chemically related steroids on their antifibromatogenic activity.—Antifibromatogenic activity is greatly enhanced by the short side chain in the 17 position: Both progesterone and desoxycorticosterone were more active than testosterone. The double bond in ring I which is essential for progestational and cortical activity seems to be nonessential for antifibromatogenic activity: Dihydrotestosterone was no less active than testosterone. Other steroids, which do not occur naturally, are now being tried as to their antifibromatogenic activity.

23. SEX HORMONE EXCRETION IN NEOPLASTIC DISEASE. IRA T. NATHANSON. (Laboratories of the Harvard Cancer Commission, Boston, Mass.)

Functioning tumors of the endocrine glands, such as the gonads and the adrenals, often exhibit excessive urinary excretion levels of one or more of the sex hormones. Excretion of these hormones in patients with nonfunction-

ing tumors of these organs seldom deviates from normal unless the function of the gland is impaired or destroyed. These facts frequently make possible the diagnosis of the site and nature of certain of these tumors. On the other hand, neoplasms of organs normally stimulated by the hormones of the glands of internal secretion do not in themselves give rise to atypical excretion levels of the sex hormones. If abnormalities are apparent, they may be caused entirely by the condition or changes in the host. Studies from this laboratory indicate at present that total excretion levels of the sex hormones in patients with various forms of cancer do not vary significantly from normal. It is recognized that these excretions give no true index as to the rate of secretion, utilization, or destruction of the hormones in the body. Moreover, there is no proof that the excretion rates were normal before development of the carcinoma or that individual components of the sex hormones exist in a normal relationship. Recent data suggest that the latter point should be given serious consideration. Normal excretion levels, therefore, do not indicate that the sex hormones are not involved in the etiology of certain tumors. Detailed studies on patients with different types of neoplastic disease will be presented to amplify the above theses.

5. SOME EFFECTS OF HYPOPHYSEAL TUMORS INDUCED BY TREATMENT WITH ESTROGENS. WARREN O. NELSON. (Department of Anatomy, Wayne University College of Medicine, Detroit, Mich.)

Ninety-one tumors of the hypophysis, ranging in weight from 105 to 412 mgm., have been obtained in rats which had received 50 to 100 gamma of diethylstilbestrol or estradiol benzoate daily for periods of 10 to 15 months.

These tumors appear invariably in animals treated for a year or more, are chromophobic in type, and are always fatal to the host. They appear to be benign since no characteristics of malignancy have been observed and efforts to obtain successful transplants have failed.

Although growth of the tumor is progressive during the first 10 or 11 months of treatment, usually no evidence of serious effects is apparent during this time. The animals show the inhibition of growth and mild anorexia which is characteristic of animals that have been injected for considerable periods of time with estrogenic hormone, but they generally exhibit no unusual symptoms until treatment has progressed for at least 12 months and the tumor reached a weight of at least 150 mgm. When symptoms do appear they are dramatic, reach a rapid climax, and usually result in death of the animal within 15 days. The first evidence of the developing syndrome is a tilting of the head toward one side and increasing defects of vision. This is followed by signal disturbances in the vestibular mechanisms, voluntary ataxy, spastic weakness, nystagmus, and defective postural fixation. As a rule death occurs suddenly, but in some instances the decline is progressive over a period of several days.

Examination by serial section of the entire brain and tumor has been carried out in 15 animals. Various neurological technics have been used to establish comparisons with the normal brain and pituitary.

The tumors usually show a bilobed condition with one side encroaching further into the brain. The posterior lobe becomes flattened and attenuated and as a rule is pushed forward as well as upward. The interglandular cleft generally is dilated and filled with colloid. The invasive process of the tumors does not involve materially the hypothalamic or thalamic areas. Very little change has been seen in the nuclei associated with the tracts entering the posterior lobe and metabolic studies have shown that the control of water balance is not disturbed materially. The tumor intrudes only partially upon the optic tracts and the evidence indicates that the visual disturbances do not arise from pressure in this area.

The tumors usually push upward in the mesencephalic and metencephalic areas and exert destructive pressure upon such structures as the cerebral peduncles, the mesencephalic and pontine nuclei and fiber tracts. The vestibular and cochlear nuclei usually are involved and show degenerative changes. The ventricular system regularly shows some degree of dilatation throughout. The various changes which have been observed throughout the brain appear to result from the effects of either direct mechanical or increased intracranial pressure. Thus far the studies have not revealed the involvement of other processes.

8. THE INFLUENCE OF ESTROGEN ON EXPERIMENTAL EPIDERMAL METHYLCHOLANTHRENE CARCINOGENESIS IN MICE. F. X. PALETTA. (Department of Surgery, University of Virginia, Charlottesville, Va.)

The influence of estradiol benzoate in epidermal methylcholanthrene carcinogenesis was studied in virgin female mice of inbred Swiss stock. The mice were 3 months of age and divided into 2 groups of 40 each. In the first group, the hair over the abdomen was cut short with scissors and 3 drops of estradiol benzoate in the form of 0.01 per cent solution in chloroform were painted on the abdominal skin every 4 days (37.5 rat units). Eighteen hours following the estrogen application a 0.3 per cent solution of methylcholanthrene in benzene was painted on an area of skin about 5 mm. in diameter at the interscapular region. The second group of mice represented the control series. They were painted with 0.3 per cent methylcholanthrene in benzene at the same time as the first group. No estrogens were applied to the control group. When the tumor was thought to be malignant, biopsies were taken and microscopic sections made. The criterion used for diagnosis of squamous cell carcinoma was the presence of neoplastic cells invading muscle.

The results indicate that application of estradiol benzoate to female Swiss mice accelerates the transformation of benign tumors into malignant tumors and shortens the induction period of methylcholanthrene skin carcinogenesis.

11. EXPERIMENTALLY INDUCED ENDOCRINE IMBALANCE AND ATYPICAL GENITAL CONDITIONS IN MICE.* CARROLL A. PFEIFFER. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

An endocrine imbalance between the hypophysis and the ovary was established in female mice by grafting

testes from litter mates at birth. This caused a constant production of estrogen which induced great hypertrophy of the genital tract. The hypertrophy involved all elements in the middle portion of the oviduct, tremendous hyperplasia of the endometrial glands which often resulted in the glands entering and even extending through the myometrium, formation of hyalinized areas in the uterine horns and cervix, and heavy cornification of the epithelium of the vagina and lower portions of the cervix. The epithelium of the cervix and localized areas of the uterine glands underwent metaplasia. Growths, possibly adenomatous, appeared near the cervix in 2 cases. The ovaries had a prematurely senile appearance and usually contained numerous luteum-like cells which were often organized to resemble corpora lutea. A few developing follicles were always present and ovulation sometimes occurred, producing pale atypical corpora lutea. All the other effects of large injections of estrogen, such as mammary gland development, pubic ligament formation, hyperossification, and hypophyseal hypertrophy also occurred. It is therefore concluded that any of the effects produced by the injection of large amounts of estrogen may be brought about by endogenous hormone provided an adequate endocrine imbalance is established.

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26. STEROID EXCRETION IN CANCEROUS AND NONCANCEROUS PERSONS. GREGORY PINCUS. (Physiological Laboratories, Clark University, Worcester, Mass.)

The urines of patients having various malignant growths were collected before and after the injection of estrone. Patients under the same hospital regime but suffering from other diseases contributed control urine specimens. As additional controls specimens from normal, healthy persons were used. The urines were separated into 2 main fractions: neutral and phenolic. The former was divided into nonketonic, ketonic alcoholic, and ketonic nonalcoholic portions. Cholesterol determinations were made of the nonketonic fractions, and the Zimmerman color titer was obtained for the ketonic fractions. The phenolic material was divided into: (1) a "strong" phenolic portion partitioned into 0.3 M Na_2CO_3 from benzene (estriol fraction); (2) a "weak" phenolic portion partitioned into benzene after washing with 0.3 M Na_2CO_3. The latter portion (2) was subdivided into a ketonic (estrone) and a nonketonic (estradiol) fraction. On the basis of these data a description of the steroid (particularly the estrogen) economy of cancerous and noncancerous persons will be attempted.

20. SEX PRECOCITY, VIRILISM, AND ADRENAL CORTICAL TUMOR. J. P. PRATT.* (Detroit, Mich.)

Three cases of sex precocity and virilism are presented. The first had a cortical adrenal carcinoma. The second had a sarcoma of the cheek which metastasized to the adrenal. The third had no hypertrophy or tumor of the adrenal.

Case 1.—F.P., age 2 years, 9 months, on April 3, 1937, was large for her age. She had a large clitoris and pubic hair. The muscular development was masculine. Her nose was large and bulbous. The deep voice was startling. Osseous development was 3 years in advance. Usual laboratory tests were within normal limits. Two bioassays of urine on separate occasions were negative for androgen and estrogen. At operation, January 15, 1938, ovaries and left adrenal were normal. Right adrenal contained a well encapsulated tumor 5.5 × 4.5 × 5.3 cm. The tumor was removed. Pathological diagnosis was cortical adrenal carcinoma. Following operation virilism developed no further. The patient has developed normally to the present time.

Case 2.—D.G., age 4 months, presented a small tumor on her cheek which was removed at 13 months. Pathological diagnosis was sarcoma. The lesion recurred 7 months later. Virilism developed after operation. Usual laboratory tests were within normal limits, 1,510 rat units of theelin in 24 hours. At laparotomy, age 20 months, the pelvic organs and right kidney were normal. A mass the size of a lemon found above the left kidney was inoperable. Metastases developed in the lungs and the child died at the age of 25 months. At autopsy a fibrosarcoma was found at the site of the original lesion and in all metastases.

Case 3.—D.B., age 8 years, showed virilism and retarded growth. From age 3 to 5 she grew only 2 inches. At 7 years the pubic hair developed, also hair on arms and legs. She was precocious in school. Her musculature was masculine and excessively developed. Labia and clitoris were large. The usual laboratory tests were within normal limits. Bioassay of urine was negative for estrogen and androgen. Osseous development advanced 1 to 2 years. Exploratory operation showed no abnormality of the pelvic organs. The adrenals were normal. Subsequently there was no advance in virilism. Her feminine development has been normal.

Comparison of these 3 cases shows similar symptoms of sex precocity and virilism associated with widely differing conditions of the adrenals.

* In collaboration with R. L. Schaeffer.

2. EFFECT OF FOSTER NURSING ON INDUCTION OF MAMMARY TUMORS IN MICE WITH ESTROGENS. MICHAEL B. SHIMKIN and HOWARD B. ANDERVONT. (National Cancer Institute, Bethesda, Md.)

Foster nursing of mice of a strain with a high incidence of mammary carcinoma (C3H) by mice with a low incidence (C57 black or C) reduces notably the incidence of mammary tumors in both males and females receiving large doses of estrogen. Foster nursing of mice of a strain with a low incidence of mammary carcinoma (C) by mice with a high incidence (C3H) increases notably the incidence of tumors of the breast in male and female mice treated with estrogen.

Mice	Nursed by	Sex	Number	Tumors	Tumors, per cent
C3H	C3H	M	44	28	63.6
C3H	C57, C	M	74	10	13.5
C	C	M	94	0	. . .
C	C3H	M	80	21	26.2
C3H	C3H	F	31	29	93.5
C3H	C57, C	F	32	6	18.7
C	C	F	54	1	1.8
C	C3H	F	31	20	64.5

The genetic and hormonal factors in the genesis of mammary tumors in mice create a suitable substratum for the carcinogenic action of the factor(s) transmitted through the milk.

7. INTERSTITIAL CELL TUMORS OF TESTES INDUCED WITH STILBESTROL IN MICE. MICHAEL B. SHIMKIN and HUGH G. GRADY. (National Cancer Institute, Bethesda, Md.)

Subcutaneous implantation of pellets of stilbestrol in cholesterol produces a notable hyperplasia of the interstitial cells of the testes in mice of strain C. In 6 to 12 months, about one-third of the animals (43 tumors in 141 animals) develop frank testicular tumors, some of which metastasize. Testicular tumors of the interstitial cell type are also induced in mice of strain A and c^e, but not in mice of strain C57 black, I, or C3H. Foster nursing exerts no influence on the appearance of testicular tumors.

Slides are presented of the gross and histologic appearance of these induced testicular tumors in mice.

24. SPECTROSCOPIC STUDY OF TRIPHENYLETHYLENE IN THE NORMAL AND CANCEROUS TISSUES OF MICE TREATED WITH TRIPHENYLETHYLENE. L. STRAIT, E. McCAWLEY, and I. PERRY. (University of California, Berkeley, Calif.)

The physiological relationship of the breast to the female generative system has long been obvious. The pathological relationships are still emergent. Because of the obscurity regarding the role of estrogens in the genesis of breast carcinoma, we undertook to study spectrographically one synthetic estrogen, triphenylethylene, in the tissues of mice treated with this substance by repeated injection at a site distant from the breast until carcinoma of the breast developed.

Using the absorption spectrum, triphenylethylene can be detected in the tissues in amounts which are of the order of one-half of the estrogenic and 1/600th of the carcinogenic dose.

From a study of the tissues of 20 C_3H male mice with breast cancer subsequent to treatment with triphenyl ethylene, 4 significant observations were made:

1. Triphenylethylene was not present in the breast of control females 2½ days after a single estrogenic dose, while the mouse was in estrus.

2. Triphenylethylene was present in the hyperplastic, chronically treated breasts of mice with cancer in another breast.

3. In the chronically treated breast there were also present "new absorption bands." These bands are suggestive of a molecular structure possibly related to triphenylethylene.

4. Triphenylethylene was not present in the breast cancer itself, nor were the "new absorption bands."

These observations suggest that the chronically treated, hyperplastic precancerous breast disposes of triphenylethylene differently than does normal or tumor tissue from the breast. Such chemical changes may be causally related to the precancerous biological state of the tissue. Breast cancer induced by triphenylethylene does not continue to metabolize triphenylethylene.

22. EXCRETION OF ESTROGENS AND ANDROGENS IN BREAST CARCINOMA IN VARYING CONDITIONS OF HORMONE ADMINISTRATION. HOWARD C. TAYLOR, JR. (Memorial Hospital, New York, N. Y.)

Work on the urinary excretion rates of estrogenic and gonadotropic hormones in cases of breast disease was begun at the Memorial Hospital about 8 years ago. Attention was first directed to the condition commonly termed "chronic cystic mastitis," because in patients with this disease some clinical signs at least suggestive of an endocrine disorder are often present. The total estrogen excretion has now been measured throughout the menstrual cycle of 27 patients with chronic cystic mastitis and also of 10 normal women. These studies have failed to disclose anything characteristic of the curves of urinary estrogen excretion in the type of chronic mastitis called adenofibrosis and characterized by diffuse fibrosis of the breast and by painful premenstrual engorgement.

In the type of breast disease associated with nonpuerperal lactation some irregularities in excretion are perhaps present, but these are compatible with the menstrual abnormalities also present and are not specific for the breast disease itself.

Hormone excretion in breast cancer was first studied on the basis of a series of discontinuous random specimens. These included 20 from young menstruating women, 27 from young women with amenorrhea, and 10 from women in the menopause. When compared with an approximately equal number of women without cancer, no characteristic differences were discernible as regards either total 17-ketosteroid or total estrogen excretion.

Although differences in spontaneous rates of excretion of estrogen and 17-ketosteroids seem not to be present in patients with breast cancer, it is still possible that more refined methods of study might yield evidence of a characteristic disturbance in steroid metabolism. One method of investigation seemed to us a study of the effects of overloading the system with administered hormones in the hope that the reaction of the patient with cancer might be different from that of the normal person.

Two women were selected of approximately the same age and each with essentially normal menstrual cycles. Both submitted for study continuous urine specimens for 7 successive months. In each case the first month was devoted to a measurement of spontaneous estrogen and 17-ketosteroid excretion, and subsequent months to

a study of the effects of the injection of estrone, progesterone, and testosterone propionate. Injections were given to the 2 subjects on corresponding days of their respective cycles. Each hormone was administered during 2 successive months, the injection being so timed that during the first of the pair of months devoted to a given hormone it was administered before the time of ovulation; during the second month, after ovulation had occurred.

The results of these studies led only to the following observations.

1. The tendency of the cancer patient to respond to the injection of either testosterone or progesterone before ovulation by alteration in the length of her cycle was in striking contrast to the normal patient. It cannot be said that this difference in response is characteristic of breast cancer. The observation of the effects on cycle length of critical amounts of several steroid hormones appears, however, possibly a practical method of studying the reproductive function in the human female.

2. The effects of hormone administration on hormone excretion rates showed nothing strikingly different in the 2 women. Differences appear in our charts but the experiment would require much repetition before these variations could be set up as typical of a physiologic disorder characteristic of breast cancer.

21. EXCRETION OF HORMONES BY PATIENTS SUFFERING FROM TESTICULAR TUMORS. G. H. TWOMBLY. (Memorial Hospital, New York, N. Y.)

Two types of gonadotropic hormones occur in the urine of men suffering from teratoma of the testis. These are the chorionic type of gonadotropin, which appears to be similar in every way to the material found in the urine of pregnant women, and the follicle-stimulating hormone, which gives reactions similar to those produced by postmenopausal or castrate urine. These 2 types of gonadotropic hormone can be distinguished by the reaction they produce in the ovaries of injected infantile female mice. The chorionic type of hormone produces the ripening of a few large follicles with prompt formation of corpora lutea in larger doses. The follicle-stimulating hormone causes the ripening of many or all the follicles with only an occasional corpus luteum in very high dosage.

Since 1936 the urine from 135 patients has been studied. Sixty-three of this number were known to have active disease when the tests were made. Of these 38 showed the chorionic type of hormone, of whom 32 are dead, 4 alive with disease, and only 2 apparently cured. In no case have we seen this type of hormone present in the urine of a normal man or a patient cured of his testicular cancer. The follicle-stimulating hormone, on the other hand, may be present in cured or apparently cured cases as well as in those with active tumor. We believe the first to be a product of the tumor, the second a product of the pituitary gland.

We may conclude from our observations that the pregnancy or chorionic type of gonadotropic hormone appears in the urine of men only when active teratoma tissue is present in the body. The finding of this material usually means that the tumor will be resistant to irradiation and that the patient will die soon. There is some evidence to suggest that this type of hormone is produced by a kind of tissue usually referred to as embryonal adenocarcinoma.

4. THE TUMORIGENIC ACTION OF STEROID HORMONES WITH EMPHASIS ON UTERINE FIBROIDS. LUIS VARGAS. (Department of Embryology, Carnegie Institution of Washington, Baltimore, Md.)

If we attempt to summarize the tumorigenic action of steroid hormones, we can say that only *estrogens* are tumorigenic. In general, all the various estrogens are tumorigenic but the esterified and synthetic estrogens are more active than the free natural ones. However, when the latter are used in pellet form and in subcutaneous implantation they become very active and induce a pronounced tumoral reaction, as several workers have shown. If we attempt to summarize the pathological reactions produced by chronic stimulation of estrogens we must accept as the principal ones *hyperplasia* and *fibrosis*. Both phenomena vary in importance according to the species, and they range from simple adenomata to malignant proliferations, and from simple fibrosis to infiltrating fibroids. Those phenomena, hyperplasia and fibrosis, sometimes occur simultaneously in the guinea pig, as we know from the work of Nelson, Lipschütz and Iglesias, and Moricard and Cauchoix. Then one animal can show the interesting combination of having, on the uterus, internal adenomatous hyperplasia and external fibroid tumoral reaction. It is possible to accept that the latter reaction originated especially from the mesothelium. Consequently, the guinea pig exhibits a double and peculiar reaction: hyperplasia-fibrosis of uterine epithelium and of mesothelium. However, epithelium and mesothelium have a *specific differential sensitivity for estrogens* completely opposite when we compare them. So, while the epithelial uterine sensitivity is increasing toward the cervix-vagina, the mesothelial uterine sensitivity is increasing toward the tubes, and also toward the kidneys, stomach, and spleen. The different sensitivity determines the topographic distribution of the fibroid tumors. Thus their distribution in the genital tract of the castrated guinea pig is about 100 per cent in the tube-cornu region; 57 per cent in the middle cornu region; 21 per cent in the cervix; and 7 per cent in the vagina. Furthermore, this graduated sensitivity of the mesothelium will determine also the genesis and development of uterine fibroids, because the threshold will be lower on the tube-cornu region and higher on the vagina. In fact, the fibroid tumoral reaction begins chronologically at the tubal end and descends little by little, according to the length of treatment, through the vagina. (The high incidence of fibroid reaction in the tube region is artificially increased by the operative trauma of castration.) It is very rare to observe fibroid tumors in the myometrium or in the tunica propria of the uterus. Nevertheless, Lipschütz and his associates have described these intrauterine tumors and have discussed the probable participation of the uterine vascular endothelium.

Some of the uterine fibroid tumors are *para-uterine*. These are the tumors of the mesometrium, beside the uterine wall, which are really "intraligamentary tumors." They arise alongside of the uterus in relation to the mesometrial blood vessels but afterwards, because of their growth, they show a tendency to include the mesometrial blood vessels, to join the uterine wall, and to fuse together. So these mesometrial tumors do not exhibit the pedunculated axial connection with the myometrium which is typical in the other uterine fibroids. We have seen that hypophysectomy does not influence the fibroid uterine reaction, either as to genesis or topographic distribution. But because of the influence of hypophysectomy on the development of the mammary gland, we have observed hypophysectomized animals without mammary glands at all and with a good deal of fibroid uterine reaction. This might be interesting for explaining the different pathologic behavior of mammary gland and uterus in the woman.

If we compare the experimental observations of fibroids with the human clinical observations, we can see some similarities. The woman shows also the simultaneous reaction of external uterine fibroids with internal adenomatous hyperplasia of the endometrium. Fibroids are almost always on the surface of the corpus uteri, exceptional on the cervix, and absent on the vagina. The parauterine intraligamentary fibroids, the most frequent extragenital fibroids of the woman, are presumably homologous with the mesometrial intraligamentary tumors above mentioned. On the other hand, as it is well known, adenoma and cancer are almost always on the cervix uteri. Therefore, we believe that not only the epithelium of the genital tract can have a differential sensitivity to estrogens, but also the mesothelium.

13. ADRENAL CORTICAL TUMORS AND THEIR SYNDROMES IN MICE.* GEORGE W. WOOLLEY. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine)

Tumors of the adrenal cortex have been studied in man. Excellent studies have been made relating these tumors to syndromes characterized for the most part by unusual or excessive masculinization and in a few cases by unusual feminization of the individual. Tumors of the adrenal cortex have been too rare and sporadic in experimental animals to be studied satisfactorily; in thousands of autopsies of mice at the Jackson Memorial Laboratory not one has been recorded. Now, with a simple technic, any number of these tumors of predictable types may be secured. The technic is: (1) to select certain specific inbred strains of mice and (2) to castrate them within 2 hours postpartum. The mice are then allowed to mature normally.

Most classifications of tumors of the adrenal cortex include hyperplasias, nodular and diffuse; adenomas; and carcinomas. The extreme classes, at least, appear with a high frequency in both males and females of some strains of mice. Hyperplasia, which occurs in 100 per cent of the individuals of the Jackson Laboratory dilute brown strain, is paralleled by feminization of both male and female. Carcinoma, which appears in a high percentage of animals of the Jackson Laboratory extreme dilution strain, leads to masculinization of both males and females. Further proof of the latter comes from tumor transplantation studies. Only slight adrenal change and only slight sexual stimulation follow castration of some strains such as the Jackson Laboratory C57 black strain.

All the tumors described originated in the outer zones of the cortex, the carcinomas in regions of hyperplasia and often following an original feminization of the individual.

Many questions are still to be answered. What is involved in the strain difference? By what process does gonad removal cause the appearance of the tumors? Why should the type of sexual stimulation be related to the type of tumor growth? How can these tumors be rendered least harmful? Work is now in progress which it is hoped will help to answer these and other questions.

* Cooperative study with Elizabeth Fekete and C. C. Little. Supported in part by a grant from the Josiah Macy, Jr., Foundation and by grants from the National Cancer Institute and The Jane Coffin Childs Fund for Medical Research.

16. THE EFFECTS OF PROLONGED ESTROGEN ADMINISTRATION ON SPONTANEOUS MAMMARY FIBROADENOMAS IN THE ALBANY STRAIN RATS. A. W. WRIGHT and J. M. WOLFE. (Departments of Pathology and Anatomy, Albany Medical College, Albany, N. Y.)

The present study was undertaken to determine the effect of the prolonged administration of estrogen on the growth and morphology of the spontaneous breast tumors which appear in female rats of the Albany strain. This strain is unique in that fibroepithelial tumors, chiefly fibroadenomas, occur spontaneously in the mammary glands of an unusually large number of females, and with close inbreeding the incidence of these tumors is increasing.

Thirty-eight rats with small spontaneous tumors were castrated and biopsies of the growths made. In general these were typical fibroadenomas, although 2 fibromas, 1 adenoma, and 1 cystadenoma were also found. Daily estrogen administration was begun 1 to 5 days after castration, in general 200 rat units of estradiol benzoate in sesame oil being employed. Injection periods varied from 3 to 272 days, 27 animals receiving the hormone for more than 30 days.

The rate and degree of growth appeared not to be influenced by the injections. Histologically, the usual changes were those of epithelial hyperplasia in the duct and acini, pronounced secretory activity, and later exhaustion of the secreting cells and cyst formation.

The most striking changes in these tumors occurred in 13 animals of 22 which had received injections for 50 or more days. Foci of abnormal proliferation of epithelium were found in these tumors. In some of these cases this abnormal proliferation strongly suggested early cancer.

These atypical foci did not occur generally throughout the tumors of which they were a part but formed minute,

circumscribed, usually nodular foci. Their histological characteristics varied. In some of the ducts epithelial cells were irregular in size and shape, often contained mitotic figures, and tended to form a thick layer which projected into the lumen. In others the process had proceeded to the point where the epithelial cells nearly filled the lumen, resembling duct carcinoma in the human breast. Occasionally large medullary masses or sheets of atypical epithelial cells were found. In a few cases cord-like masses of atypical epithelial cells invaded the stroma or formed a scirrhous type of growth which strongly resembled scirrhous carcinoma of the human female breast. One tumor was a bulky papillary cystadenomatous mass which, in a human breast, would be called a cystadenoma. None of these tumors formed remote metastases and a decision as to their truly malignant character must await further study.

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

BERGMANN, F. [Daniel Sieff Research Inst., Rehovoth, Palestine] ON THE MECHANISM OF TUMOR PRODUCTION BY CHEMICAL AGENTS. Cancer Research, 2:660-663. 1942.

The following principles for tumor production by chemical agents are hypothetically established:

The form and size of a carcinogenic hydrocarbon and of its related heterocyclic compounds determine its activity. Every substance which imitates more or less closely a given parent structure resembles it also in its blastomatogenic action.

In the living cell, the carcinogens are adsorbed by an acceptor possessing a definite adsorption area. This sets an upper limit for the dimensions of the active compounds. A lower limit is given by a decrease in adsorbability with decreasing size of the molecule.

All carcinogens are conceived as parts of an "ideal carcinogenic structure."

A carcinogen can be inactivated by additions which prevent its proper adsorption. The mechanism of this hindering effect is discussed.—Author's summary.

BIELSCHOWSKY, F., and GREEN, H. N. [Univ. of Sheffield, England] 2-AMINOFLUORENE AS GROWTH INHIBITOR FOR BACTERIA AND RATS. Nature, London, 149:526-527. 1942.

In an effort to discover common mechanisms responsible for growth-inhibition of tissue cells and bacteria, colloidal preparations of various carcinogenic compounds were tested for possible bacteriostatic action. No very significant results were obtained with 1,2,5,6-dibenzanthracene, 3,4-benzpyrene, or methylcholanthrene, but it was found that 2-aminofluorene, when dissolved in nutrient broth, exerted a bactericidal or strong bacteriostatic effect on *Staphylococcus aureus* and certain other microorganisms. The corresponding acetyl compound—already known as an insecticide and recently stated by Wilson, DeEds, and Cox (Cancer Research, 1:595-608. 1941) to be carcinogenic—was further shown to produce a considerable inhibitory effect on the growth rate of rats. It was therefore decided to investigate related fluorene compounds, and the following were synthesized and tested: 2-aminofluorenone, 2-aminofluorenol, 9-aminofluorene, and the corresponding acetyl compounds. The last were used in rat feeding tests, and the compounds with a free amino group for bacterial tests *in vitro*. Of all the substances mentioned, only 2-acetylaminofluorene had any significant growth-inhibiting action in rats (effect shown in graph), and the action of the related compounds on bacterial growth was similar, complete bacteriostasis occurring only in the case of 2-aminofluorene.

The authors regard these results as indicating the importance of the two reacting hydrogen atoms in the 9- position, in combination with an amino group in the 2- position, in determining the growth-inhibiting and possibly the carcinogenic activity of 2-aminofluorene.

Note: There is an error in the legend attached to the graph.—A. H.

WEIL-MALHERBE, H., and WEISS, J. [Royal Victoria Infirmary, and King's Coll., Univ. of Durham, Newcastle-upon-Tyne, England] REVERSIBLE QUENCHING BY OXYGEN OF THE FLUORESCENCE OF POLYCYCLIC HYDROCARBONS. Nature, London, 149:471-472. 1942.

The fluorescence of 3,4-benzpyrene when dissolved in tetralin was found to be 5 times as powerful as when dissolved in hexane. The difference results from the oxygen content of the solvents. If O_2 is pumped off, the fluorescence of the two solutions is practically identical. This inhibition of fluorescence by O_2 is called "quenching." Even in the absence of O_2 self quenching occurs above a certain concentration and it is suggested that this results from dimerization of the hydrocarbon molecule.

The percentage quenching of fluorescence by O_2 at 1 atmosphere pressure in ethanol solutions containing 0.01 mgm. per cc. of hydrocarbon is: for chrysene, 85%; pyrene, 87.7%; 1,2-benzanthracene, 86.3%; 9,10-dimethyl-1,2,-benzanthracene, 86.7%; 1,2,5,6-dibenzanthracene, 88.2%; 3,4-benzpyrene, 91.7%; and 20-methylcholanthrene, 86.6%.—I. H.

BOWEN, E. J. [Physical Chemistry Lab., Oxford, England] REVERSIBLE QUENCHING BY OXYGEN OF THE FLUORESCENCE OF POLYCYCLIC HYDROCARBONS. Nature, London, 149:528. 1942.

In a comment upon the work of Weil-Malherbe and Weiss (see preceding abstract) the author says:

. . . . "The fluorescence of naphthalene was found to be quenched at least as powerfully as the 3:4-benzpyrene to which attention is directed. . . . . The general relations between fluorescence quenching and actual photo-oxidation by oxygen appeared to be of a very complex nature."

—I. H.

WEIZMANN, M., and BOGRACHOV, F. [Hebrew Univ. Jerusalem, Palestine] DERIVATIVES OF 1'-AZA-3:4-BENZPYRENE. J. Chem. Soc., 377. 1942.

The synthesis of two derivatives of 1'-aza-3,4-benzpyrene from 3-aminopyrene and ethylacetoacetate is described.—E. L. K.

Hormones

EISEN, M. J. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] THE OCCURRENCE OF BENIGN AND MALIGNANT MAMMARY LESIONS IN RATS TREATED

WITH CRYSTALLINE ESTROGEN. Cancer Research, 2:632-644. 1942.

Crystalline estradiol dipropionate (1 to 20 mgm.) implanted in 134 rats of known lineage induced chronic diffuse alterations in the genitomammary system of both sexes, among which a fibrocystic mammary disorder preeminently of a benign nature, was the outstanding manifestation. By means of reimplantation of estrogen in those animals judged by vaginal smear or the position of the testes to have utilized completely the earlier dose, the largest number were maintained constantly under estrogen influence throughout their lives. The lesions in the breast developed progressively as a sequence of events characterized by considerable variability but occurring in all animals. The initial manifestations were present in the epithelium and consisted in proliferation, chiefly of the ducts; signs of functional activity by the cells; microscopic, followed by macroscopically visible enlargement of the lumina as a consequence of accumulated secretions; and exhaustion of cellular activity with subsequent evidence of disintegration of the cells or reversion of the epithelium to a resting stage. These lesions appeared responsible for the succeeding changes in the stroma. Proliferation of the fibrous tissue about cystic ducts was followed by varying degrees of diffuse fibrosis in all rats surviving 180 days or more. This gave rise to contracted and distorted configurations of the persisting mammary elements, and resulted ultimately in the production of a state comparable in many respects with human chronic cystic mastitis. With the later development of somewhat more atypical foci of secondary proliferation of the duct epithelium in the involved zones this comparison appeared even more appropriate. These proliferations occurred in 6 rats and lesions interpreted as mammary adenocarcinoma in 2 of 103 animals surviving 242 days, the minimal time at which these changes were evident. Of associated changes in other tissues, a single example of squamous cell cancer of the cervix was of interest. The manifestations which occurred in animals that received estrogen have not been observed in untreated control rats.

While the etiologic agent which produces in the rat a chronic, benign, fibrocystic mammary lesion was a slow, constant absorption of estrogen from a focus containing the crystalline material, it is concluded that additional factors of an unknown nature, possibly acting locally, are more directly concerned with the origin of the late secondary epithelial proliferations and true neoplasia.—Author's abstract.

HAAGENSEN, C. D., and RANDALL, H. T. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] PRODUCTION OF MAMMARY CARCINOMA IN MICE BY ESTROGENS. Arch. Path., 33:411-442. 1942.

An experimental study extending over 4 years and involving 1,526 mice surviving to the minimal cancer age warrants the conclusion that estrone benzoate in oil given to the limit of tolerance to mice of pure bred strains has the following results: It increases the incidence of mammary carcinoma in the males of the high cancer strains, bringing up the incidence in the Paris RIII males to a level that is somewhat higher than that in female bred controls. It fails to increase the frequency of mammary carcinoma in female mice of the high cancer strains above the natural frequency in bred female controls. It lowers the age at which mammary carcinoma develops in the female mice of the Paris RIII high cancer strain. It does not induce mammary carcinoma in either males or females of the low cancer C57 strain. The histological evidences of estrogen stimulation in the mammae of these strains of mice parallel the effects on tumor incidence. The estrogen treatment did not induce sarcoma, lymphoblastoma, epithelioma of the cervix, or any other form of extramammary cancer in the mice. Hereditary factors are dominant over the effect of the administration of estrogen on the incidence of mammary carcinoma.—Authors' summary.

Leukemia

DOLJANSKI, L., and PIKOVSKI, M. [Hebrew Univ., Jerusalem, Palestine] AGENT OF FOWL LEUKOSIS IN TISSUE CULTURES. Cancer Research, 2:626-631. 1942.

Maintenance of the agent of fowl leukosis (strain T1 of Engelbreth-Holm) was found possible not only in the presence of specific cell elements of normal bone marrow, but also in cultures of normal, common, spindle cells. Since leukotic agent could be maintained fully active in normal fibroblast cultures carried in serial passage for 178 days, it is considered probable that an increase of the agent occurs *in vitro*. The presence of a leukotic agent in the cell cultures causes no changes in the appearance of the latter and no alterations in individual fibroblasts.

The cell colonies were cultivated in Carrel flasks. Two kinds of experiments were carried out: (1) Normal bone marrow and normal myocardium were cultivated separately in the plasma of leukotic fowls. (2) Fibroblast colonies were bathed for 1 hour in ultrafiltrate of heart and spleen extract of leukotic fowls, and then cultivated, first, in normal plasma diluted with 2 parts of the ultrafiltrate, and later, in normal plasma diluted with Tyrode's solution. At intervals, the cultures were washed with Tyrode's solution and fed with normal plasma (method of Fischer-Parker). Both the cell cultures and the cell-free supernatant fluid from the cultures proved infective for fowls.—Authors' abstract.

POTTER, J. S., and WARD, E. N. [Carnegie Inst. of Washington, Dept. of Genetics, Cold Spring Harbor, N. Y.] MITOCHONDRIA IN LYMPHOCYTES OF NORMAL AND LEUKEMIC MICE. Cancer Research, 2:655-659. 1942.

By the use of the supravital technic, mitochondria were counted in 100 lymphocytes in the peripheral blood from each of 10 strain C58 mice in the following classifications: young normal, older normal, one spontaneous leukemia, and 4 transplantable leukemias. Tables and distribution curves are presented to show differences in number among the various classifications. Descriptions and photomicrographs of differences in size and shape of mitochondria are given. Although the size of a cell is correlated to a certain extent with the amount of its mitochondrial material, it is shown that populations of lymphocytes with the highest proportion of immature cell types have the highest mean number of mitochondria. Since individual normal and leukemic cells of the same type were not found to be different, it is concluded that the age of a cell rather than its pathogenicity determines its mitochondrial con-

tent. This agrees with Wiseman's conclusion from studies on the rabbit that the amount of mitochondrial material is indicative of the age of a lymphocyte.—Authors' abstract.

Transplantation

GREENE, H. S. N. [Rockefeller Inst. for Med. Research, Princeton, N. J., and Yale Univ. Sch. of Med., New Haven, Conn.] HETEROLOGOUS TRANSPLANTATION OF A HUMAN FIBROSARCOMA. Cancer Research, 2:649-654. 1942.

A human fibrosarcoma was successfully transplanted to the anterior chamber of the eyes of guinea pigs and has been carried by serial transfer through 14 generations over a period of 2 years. In addition, the tumor has been successfully transferred to the guinea pig's testicle.

The growths in the anterior chamber fulfill all the requisites of successful heterologous transplants. They can be transferred serially and apparently maintained indefinitely by continued passage. Growth is progressive and, in several instances, has continued for more than a year with extensive invasion of orbital tissues. Metastasis has not occurred but this is apparently a function of time, for the ability of the tumors to grow in other parts of the body was demonstrated by successful testicular transfer.—Author's abstract.

Tissue Culture

COMAN, D. R. [Univ. of Pennsylvania Med. Sch., Philadelphia, Pa.] HUMAN NEOPLASMS IN TISSUE CULTURE. Cancer Research, 2:618-625. 1942.

Various human neoplasms were maintained *in vitro* by the roller tube method of tissue culture. These tumors included carcinomas, sarcomas, and a variety of benign growths. The patterns of growth were recorded photographically and described. Benign glandular epithelial tumors formed acini after a month of culture, whereas malignant glandular epithelial tumors never formed acini. A squamous cell carcinoma produced structures *in vitro* resembling epithelial pearls. Individual neoplastic epithelial cells frequently broke away from the cell clusters and displayed ameboid movement. Such isolated cells sometimes gave rise to new colonies. An angiosarcoma formed endothelial tubes *in vitro*. These structures resembled vessels and produced lateral branches. Some neoplasms that presented a similar appearance in histologic sections behaved differently *in vitro*. These differences were noted both in the neoplastic cells themselves and also in the cells of the stroma.

The feasibility of the roller tube method for the study of a wide variety of living human neoplasms is demonstrated and its value as a method in investigative oncology emphasized.—Author's abstract.

Treatment—Radiation, Chemotherapy, etc.

KLINE, B. E., WASLEY, W. L., and RUSCH, H. P. [Med. Sch., Univ. of Wisconsin, Madison, Wis.] TUMOR INHIBITOR STUDIES. I. THE EFFECT OF PURE CHEMICAL COMPOUNDS ON TUMOR TAKES. Cancer Research, 2:645-648. 1942.

The inhibiting qualities of more than 40 water-soluble compounds were tested on the Flexner-Jobling rat carcinoma. The method of testing was a modification of that previously described by MacFadyen and Murphy. The tumor was minced, suspended in saline, and mixed with a solution of the compound to be tested. After the cells had stood for 30 minutes, rats were inoculated with 0.2 cc. of the treated suspension in the left groin and with 0.2 cc. of untreated (control) suspension in the right groin. The compounds were tested at levels which were not toxic to the animal and which did not cause hemolysis or severe crenation of red blood cells.

β-Indolebutyric acid, β-indolepropionic acid, chloretone, chloraldehyde, and sodium trichloracetate caused complete inhibition. Lysine, arginine, glycine, proline, hydroxyproline, tryptophane, urea, guanidine, sulfanilamide, sulfaguanidine, allantoin, alloxan, avidin, and malonic acid were among the inactive compounds tested.—Authors' abstract.

Tumors in Plants

WHITE, P. R., and BRAUN, A. C. [Rockefeller Inst. for Med. Research, Princeton, N. J.] A CANCEROUS NEOPLASM OF PLANTS. AUTONOMOUS BACTERIA-FREE CROWN-GALL TISSUE. Cancer Research, 2:597-617. 1942.

Sunflower plants affected with crown-gall disease produced by the bacterium *Phytomonas tumefaciens* develop primary tumefactions at the site of inoculation and secondary growths at some distance from this site. Tissues of the latter type have been isolated, shown to be generally bacteria-free, and grown in tissue culture for long periods. *In vitro* they maintained a rapid, disorganized type of growth contrasting sharply with that of corresponding tissues from uninfected plants. Implantation of viable fragments of such tissue cultures under the bark of healthy sunflower or artichoke plants resulted in the production of typical crown galls of characteristic rapid and disorganized growth but free of bacteria.

The evidence indicates that crown-gall disease in the sunflower produces in some unknown manner a profound and permanent change in the characteristics of certain cells resulting in a subsequent independent, unrestrained, unphysiologic, and potentially malignant type of development. Since these are the characteristics which are commonly used to distinguish cancerous from merely inflammatory growths in animals, it is believed that these secondary tumefactions and the tissue cultures derived from them may prove to be a valuable new avenue of approach to the fundamental problems involved in the etiology of tumors.—Authors' abstract.

Clinical and Pathological Reports

MAYO, C. W. [Mayo Clinic, Rochester, Minn.] THE CANCER PROBLEM: ITS SCOPE AND IMPORTANCE. Surg. Clin. North Am., 21:963-967. 1941.

The survival curves of patients who have undergone operations for malignant lesions of the thyroid, colon, and stomach are compared with those of the normal population. From the slopes of the curves it appears that patients who have survived 5 to 7 years after operation have about as good a chance for survival beyond this as has the normal population.—J. L. M.

Diagnosis—General

BRODERS, A. C. [Mayo Clinic, Rochester, Minn.] THE MICROSCOPIC GRADING OF CANCER. Surg. Clin. North Am., 21:947-962. 1941.

The grades of malignancy of carcinomatous neoplasms, as revealed by histological technic, are stated to be in direct proportion to their proliferative, infiltrative, metastasizing, and death-dealing capacities. The author believes that although the grading of cancer has its greatest value in prognosis, it is also of assistance in determining the most effective therapeutic procedure to be employed in specific cases.—J. L. M.

JETER, H., EPPS, C., and HART, M. S. [Oklahoma City, Okla.] THE EXAMINATION OF PARACENTRIC FLUIDS FOR MALIGNANCY. South. M. J., 35:519-523. 1942.

The examination of paracentric fluids in cases of obscure diagnosis is a satisfactory procedure and often proves of great value. The demonstration of tumor fragments is of utmost importance and justifies careful attention to the matter of concentration of adequate amounts of fresh, properly fixed specimens.—H. G. W.

PAULSON, M., and WYLER, C. I. [Johns Hopkins Hosp., Baltimore, Md.] MULTIPLE TESTS OF HEPATIC FUNCTION IN GASTROENTERIC MALIGNANCY; THE VALUE OF BROMSULPHALEIN, HIPPURIC ACID AND THE VAN DEN BERGH REACTION IN DETECTING HEPATIC METASTASIS, WITH AN EVALUATION OF NORMALITY OF THE HIPPURIC ACID TEST. Ann. Int. Med., 16:872-878. 1942.

The tests listed in the title were made on 25 patients with carcinoma of the stomach or colon who had no clinical evidence of metastasis. A correlation was found to exist between the presence of gross hepatic metastasis, as determined by subsequent operation, and the degree of bromsulfalein retention, although individual exceptions occurred. The van den Bergh reaction was not of diagnostic aid. No significant correlation was found between the presence of metastasis and the amount of hippuric acid excreted, but in all 6 cases in which no hippuric acid was found metastases were present. A second series of patients with various diseases but no apparent hepatic damage was tested, and 16 of 25 showed lowered hippuric acid synthesis although the bromsulfalein excretion was normal in 23. Extreme delicacy in tests of hepatic function may reduce their importance as diagnostic aids.—H. G. W.

Skin and Subcutaneous Tissues

NEW, G. B. [Mayo Clinic, Rochester, Minn.] TREATMENT OF CANCER OF THE FACE INCLUDING RECONSTRUCTIVE SURGERY. Surg. Clin. North Am., 21:969-978. 1941.

The following topics are discussed: sites of facial cancer; types and treatment of precancerous lesions; and lesions about the eye, of the forehead and cheek, of the scalp, and the lip.—J. L. M.

PHILLIPS, C. [Scott and White Clinic, Temple, Tex.] MULTIPLE SKIN CANCER: A STATISTICAL AND PATHOLOGIC STUDY. South. M. J., 35:583-590. 1942.

In approximately 200,000 patients seen in Texas, about 1,400 had skin cancer and in 226 cases the skin cancers were multiple. The 226 persons had a total of 704 microscopically verified skin cancers, which numbered from 2 to 23 separate lesions per patient. One hundred and thirty-six patients had 2 cancers each; 36 had 3; 22 had 4; 18 had 5; 7 had 6; while 4 to 1 each had up to 23 cancers. Of the entire 704 lesions, 19.3% were in the group of patients having 2 malignant growths each. Males predominated by about 4 to 1. One hundred and nine of the 226 persons were farmer-ranchers and therefore exposed greatly to the sun, and apparently blonds are more susceptible to the disease than brunets. With the exception of 18 on the hand, all cancers were about the head and neck. The histologic type of growth was: basal cell, 52.9; squamous cell, 44; mixed, 1.5; transitional cell, 1.4; and 4.5% of the tumors were melanoma. Emphasis is laid on the occurrence of a type of susceptibility to skin cancer, in persons with "skin-cancer-skin." The occurrence of cancer of internal organs seems to be sufficiently frequent to cast doubt on Peller's contention that skin cancer protects against other cancers.—H. G. W.

Nervous System

ADSON, A. W., and BAKER, G. S. [Mayo Clinic, Rochester, Minn.] THE SURGICAL TREATMENT OF TUMORS OF THE BRAIN. Surg. Clin. North Am., 21:979-1008. 1941.

Following a review of the surgical treatment in a consecutive group of tumors, it was observed that 70% of the tumors were situated above and 30% below the tentorium. Forty-five per cent were encapsulated; 15% of these were meningiomas, 14% pituitary tumors, and 8.6% neurofibromas. The remaining group of encapsulated tumors were cholesteatoma, ependymoma, and so forth. Including the operable gliomas, 58.1% of the tumors of the brain had been effectively treated by total or subtotal removal. The average mortality was 9%. Lesions of the posterior fossa carried a higher mortality than those situated above the tentorium.

From this discussion it appears that the condition of all patients with tumors of the brain is not hopeless, although a large number have inoperable tumors. The results inevitably will improve and the mortality rate decrease as more about the life cycle and behavior of the various groups of tumors is learned. The earlier tumors are recognized, the less will be the cerebral destruction before treatment is instituted.—J. L. M.

SMITH, W. A., and FINCHER, E. F. [Atlanta, Ga.] INTRACRANIAL TUMORS IN CHILDREN. PRELIMINARY STUDY OF 100 CASES. South. M. J., 35:547-553. 1942.

Of 100 cases of intracranial tumors in children, 86% of 87 verified cases were gliomas, and 63% were situated below the tentorium. Of 60 gliomas classified histologically, 50% were medulloblastomas, 36% were astrocytomas, and 10% ependymomas.—H. G. W.

Breast

HARRINGTON, S. W. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE BREAST WITH RESULTS OF RADICAL MASTECTOMY. Surg. Clin. North Am., 21:1063-1081. 1941.

This discussion includes the following topics: subjective symptoms, considerations in treatment, criteria for operability, and a study of the survival rates for various periods after operation.—J. L. M.

INGLEBY, H. [Woman's Med. Coll. of Pennsylvania, Philadelphia, Pa.] NORMAL AND PATHOLOGIC PROLIFERA-

TION IN THE BREAST WITH SPECIAL REFERENCE TO CYSTIC DISEASE. Arch. Path., 33:573-588. 1942.

A study of the mammary gland by means of the slicer technic, which makes especially clear the relation of simple cystic disease and fibroadenoma. The tumor consists of an overgrown duct and its branches. Often there is no cystic dilatation, partly because the periductal connective tissue has undergone abnormal proliferation or has failed to regress, and partly because the surrounding breast has not yielded to the expansion of the abnormal ducts. Each lobule of the tumor represents the expansion of one unit, that is, one branch with its subdivisions and their periductal connective tissue. The dependence of the condition on hormone imbalance is clear from the morphologic pattern. The varieties of cystic disease are the outcome of a physiologic derangement, and the changes are reversible to a much greater degree than is usually thought. —H. G. W.

WILLIAMS, I. G. [Middlesex Hosp., London] CARCINOMA OF THE MALE BREAST. Lancet, 1:701-703. 1942.

A series of 20 cases of carcinoma of the male breast is recorded: 9 patients died and of the surviving 11, 7 have lived an average of 48.4 months since treatment. The cases are classified according to clinical stages and histological grades, and 10 are analyzed to show the bearing on survival of the degree of malignancy and the extent of anatomical spread.—A. H.

Ovary

DOCKERTY, M. B., and MASON, J. C. [Mayo Clinic, Rochester, Minn.] CLINICAL FEATURES, TYPES AND GRADES OF MALIGNANT OVARIAN TUMORS. Surg. Clin. North Am., 21:1201-1210. 1941.

Ovarian tumors are divided into two groups: cystic tumors which are usually benign, and solid tumors which frequently are malignant. Certain tumors of the solid type produce hormones, and in these neoplasms functional as well as cytological differentiation of cells apparently is present. Prognosis in this group is excellent. With the exception of the dysgerminoma, primary solid carcinoma of the ovary is more serious than malignant cystic disease of the ovary. In the dysgerminoma is presented the unusual coincidence of an extremely malignant lesion associated with a good prognosis. A metastatic ovarian malignant process is a hopeless form of "cancer," in spite of the apparent ease with which the ovarian tumor may be removed.—J. L. M.

SCHILLER, W. [Cook County Hosp., Chicago, Ill.] THE HISTOGENESIS OF OVARIAN MESONEPHROMA. Arch. Path., 33:443-451. 1942.

This tumor is characterized by the fact that the cells lining the cystic cavities are of an endothelial and not of an epithelial type, and the tumor tissue contains specific structural units which duplicate the embryonic glomerulus. They are assumed to develop from misplaced remnants of the mesonephros. This can easily occur because the mesonephros develops adjacent to the hilus of the ovary. This theory is supported by the study of a benign renal tumor which presented structures analogous to the glomeruloid units of ovarian mesonephroma.—H. G. W.

TAYLOR, H. C., and GREELEY, A. V. [New York, N. Y.] FACTORS INFLUENCING THE END-RESULTS IN CARCINOMA OF THE OVARY. Surg., Gynec. & Obst., 74:928 934. 1942.

Report of a series of 138 cases of cancer of the ovary treated from 1910 to 1935, with a 5 year cure rate of 15.2%. The factor which most affected prognosis was the gross stage of the disease at the time of operation, and next was the histologic grade of the tumor, at least in the sense that the differentiated tumors of perhaps doubtful malignancy had a very much better prognosis than the others. The effect of x-ray on the percentage of 5 year cures and on survival curves was less than has been frequently stated.—H. G. W.

Urinary System—Male and Female

BARNES, M. [Ball Memorial Hosp., Muncie, Ind.] PRIMARY CARCINOMA IN A CONGENITALLY SINGLE FUNCTIONING KIDNEY. Arch. Path., 33:537-540. 1942.

A report of the only recorded case of the kind. The patient was a man of 49.—H. G. W.

PRIESTLEY, J. T. [Mayo Clinic, Rochester, Minn.] TREATMENT OF MALIGNANT RENAL TUMORS. Surg. Clin. North Am., 21:1173-1180. 1941.

Malignant tumors of the kidney are classified as adenocarcinoma ("hypernephroma"), epithelioma of the renal pelvis, Wilms' tumor, and sarcoma. The treatment and results are discussed.—J. L. M.

Oral Cavity and Upper Respiratory Tract

ERICH, J. B. [Mayo Clinic, Rochester, Minn.] MALIGNANT TUMORS OF THE MOUTH. Surg. Clin. North Am., 21:1017-1025. 1941.

Malignant tumors of the oral cavity (cheek, jaws, and tongue) are discussed under diagnosis and treatment. Fortunately, the successful management of malignant tumors of the mouth is fortified by their visibility and accessibility. Unfavorable results in the care of these lesions occasionally are unavoidable, but usually are based upon one or more of the following factors: (1) a failure of the clinician to recognize malignant disease of the mouth, with the result that much valuable time is lost before proper treatment can be instituted; (2) the application of caustic drugs, particularly silver nitrate, which may increase seriously the activity of a relatively slow growing neoplasm; (3) insufficient treatment or unsuitable forms of treatment; and (4) neglect of the regional lymphatics. —J. L. M.

HAVENS, F. Z. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE NOSE, NASOPHARYNX, AND PARANASAL SINUSES. Surg. Clin. North Am., 21:1009-1015. 1941.

Symptoms, diagnosis, and treatment are discussed. The lesions produced by carcinoma of the nose, paranasal sinuses, and nasopharynx cannot, as a rule, be readily visualized, since they may remain symptomless for a considerable time and also because the first symptoms often refer to some other structure, such as the neck, the eye, or the ear. Consequently, the physician must be alert to recognize early the symptoms and signs suggestive of malignant disease involving these structures.—J. L. M.